Na and K Fluxes in *Nitella clavata*

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ABSTRACT Na and K influxes and effluxes, membrane potentials, and cell ion concentrations of *Nitella clavata* were measured as functions of external NaCl concentrations and time. It appears necessary to conclude that active K transport into the cells as well as active Na extrusion is present, although the latter is of small magnitude and possibly is explicable as exchange-diffusion. An attempt has been made to account for the capacity of the cells to discriminate between K and Na ions and yet have fairly independent passive ion movements. This is done by proposing a model in which the permeation areas are "slit-pores" rather than cylindrical pores. The slit-pores would permit rather independent movements of ions within them so long as the pores do not tend to become saturated from both or either side. In the latter case one way movement results. The experimental results are in fair agreement with this suggested model.

INTRODUCTION

In the *Characeae* 80 to 90 per cent of the cellular electrolyte concentration is accounted for by K, Na, and Cl (1, 2). According to the simplest hypothesis proposed for the distribution of these ions, there is an inwardly directed chloride pump and an outwardly directed sodium pump, with Na influx, Cl efflux, and both K fluxes being entirely passive (3, 4). Because of the predominance of these three ions in the cell, the total Na plus K concentration is constrained to about the same value as the Cl concentration. The cellular [Cl] will stabilize at the level at which Cl leakage efflux becomes equal to the Cl pumping rate. Presumably the rate of Cl pumping is dependent on the external [Cl] and the membrane potential, but no definite information is available on this point. The Na/K concentration ratio will be determined by the external concentrations and relative permeabilities of these ions, the membrane potential, and the Na extrusion rate.

The data supporting this hypothesis have been brought together by Briggs, Hope, and Robertson (5), and, in brief, indicate that the influx and efflux

of each of these three ions are approximately in balance, whereas the electrochemical gradients favor Na gain and Cl loss by the cell. The situation may be somewhat more complex than this, however, for MacRobbie (6) has reported that a part of K influx in *Nitella translucens* was eliminated by treatment with ouabain, a specific inhibitor of Na-K transport in animal cells. Dainty (7) has likewise alluded to the possibility of Na-K–coupled pump in characeous species, but he has also pointed out that the evidence in its favor is rather scant. Perhaps of more interest is Dainty's (8) call for a more realistic approach to the treatment of ion fluxes through membranes; *i.e.*, one which does not rest upon the assumption that ion movements are independent.

In the present paper the Na and K fluxes for internodal cells of *Nitella clavata* in solutions containing variable amounts of NaCl are reported. The membrane potentials and cell ion concentrations necessary to describe the electrochemical gradients for these two ions have also been measured. An attempt is made to relate these data to a model derived from those of Mullins (9) and Rickert (10). Since, as the data below will show, the net fluxes of both Na and K are down electrochemical gradients, it seems worthwhile to look into the possibility that the interdependences predicted by the model adopted may account for the deviations of the experimental flux ratios from those predicted for independent movement.

MATERIALS AND METHODS

Materials

Internodal cells of *Nitella clavata* Kutz. (var. X033) were used. The starting stock was obtained from Dr. Vernon Proctor who originally collected the material in Cochabamba, Bolivia. Although these cells were somewhat paler than the California strain previously used (11), sodium influxes under a given set of conditions were of about the same magnitude. The culture conditions were the same as those previously described (11) except that the Na and NO_3 concentrations were reduced by 0.9 mM each. The decision to use bicarbonate in the experimental solutions (compositions given below) was based on the observation (11) that its presence resulted in enhanced Na influxes, and this was desirable for purposes of measurement. However, cells in these solutions lost chlorophyll at the rate of about 2 per cent per day, so that by 70 days after harvest most of the chlorophyll was gone and the cells began to die. There appeared to be no general correlation of the magnitude of ion fluxes with the degree of greenness except in those cases in which the chlorophyll loss was almost complete and the cells began to die. With one batch of cells the mean life span was considerably shorter than the usual 70 days, although the chlorophyll content was still appreciable; for brevity, the phrase "in poor condition" has been applied to these cells. It has been observed that cation fluxes increase markedly in cells which are within 2 or 3 days of death. Except where expressly indicated, the experiments were conducted with cells in good conditions, usually 20 to 40 days after harvest.

Experimental Conditions

The same constant conditions of light and temperature (23°C) were used throughout both the preconditioning and the experimental periods, with the preconditioning period being at least 10 days. The light intensity from 25 watt incandescent lamps was 2500 ± 200 ergs/cm² sec. for the wavelength interval 350 to 750 mμ; this corresponds to about 40 foot-candles. The control (and preconditioning) solution contained 1 mM NaCl, 1 mM $NaHCO_3$, 1 mM $CaCl_2$, 1 mM $MgSO_4$, and 0.05 mM K_2SO_4 and will hereinafter be referred to as the 2 mM Na solution. Solutions having different concentrations of NaCl will be referred to by similar abbreviations; for example, the 40 mM Na solution is a 2 mM Na solution plus 38 mM NaCl. Solutions containing less than 2 mM Na were prepared by use of a stock solution of $Ca(HCO_3)_2$ made by dissolving $Ca(OH)_2$ in CO_2-saturated water, after which the excess CO_2 was removed by aeration. As an example, the 0.1 mM Na solution contained 0.1 mM NaCl, 0.5 mM $Ca(HCO_3)_2$, 0.5 mM $CaCl_2$, 1 mM $MgSO_4$, and 0.05 mM K_2SO_4. The pH values of all solutions fell within the range 8.0 to 8.5.

General Experimental Methods

Most of the methods are very similar to those used previously (11). In brief, internodal cells were separated from the neighboring internodal cells by cutting away the latter; still left attached to the two ends of each internodal cell were the much smaller nodal cells (for details see Effluxes below). Vacuolar sap samples were obtained with 5 μl capillary tubes and analyzed for Na and K by flame photometry, and for Cl by a coulometric method. Membrane potentials were measured with a type 1230A General Radio Co. electrometer. The abbreviation pmole is for picomole (10^{-12} mole).

Influxes

Influx measurements were carried out with Na^{24} and K^{42}. Exposure of internodal cells to the radioisotopes was for 1 to 3 days, followed by a 2.5 hour rinse in non-tracer solutions of the same composition (3 changes of solution). The internodal cells were then radioassayed directly under a Geiger-Muller end-window tube, with the detection geometry being taken into account. With this procedure the error in the radioassay of a cell might be as high as 10 per cent for Na^{24} and 5 per cent for K^{42}. Variation among cells in influxes was about 40 per cent (coefficient of variation 0.4). Each influx determination involved 5 to 15 cells; in most cases 8 to 10 cells were used.

With the above rinsing procedure Na^{24} uptake was found to be proportional to the time spent in the tracer solution except in those cases in which cell permeability characteristics were changing as a result of the experimental treatment. The K^{42} uptake curves sometimes exhibited a positive fast-fraction "shoulder," sometimes a negative one, making these results less certain than those for Na^{24}. In the influx calculations the total uptakes and the total time intervals were used. Corrections for the back fluxes of tracer ions were negligible.

Effluxes

In the efflux determinations, as made with Na^{22} and K^{42}, a gamma well-type scintillation counter was used for Na^{22} and the G-M tube for K^{42}. The uptake period was about 2 weeks in the former case and 20 hours in the latter. In both cases it was necessary to rinse the cells 2 days before apparently constant effluxes were attained. This was to be expected since the small nodal cells at each end of the internodal cell would have considerably higher specific activities of the radioisotope than would the internodal cell itself. The magnitude of this factor was not appreciated until after the completion of the flux studies, when a microscopic examination indicated that attached to each internodal cell were 300 to 400 nodal cells with a mean diameter of roughly 100 μ. The nodal cells add about 1.3 per cent to the internodal cell volume and 13 per cent to the surface area; the specific activity of the radioisotope in these cells would be about 10 times that in the internodal cell, if the influxes and ion concentrations are the same. After a 2 day rinse, the nodal cell efflux would still account for about 50 per cent of the total efflux, for effluxes in the range 0.1 to 0.2 pmole/cm^2 sec. The half-times would be at least 6 days. On the same basis, the influxes would be only 12 per cent higher than the true values.

With the 2 day rinse, K effluxes were actually found to be considerably higher than the expected values as calculated from net efflux and unidirectional influx data, thus tending to confirm the suspected importance of the efflux contributions of the nodal cells. The K efflux values reported in this paper may be as much as twice the true values for internodal cells, but since this estimate involves a number of assumptions, it cannot be treated as an accurate correction factor. The Na^{22} efflux measurement of principal concern (Fig. 1) was made after 4 weeks of soaking in non-tracer solution, but since the calculated half-time for Na^{22} loss from nodal cells would be about 4 weeks (same basis as above), the reported Na efflux values may be about 50 per cent higher than the true ones.

The coefficient of variation of Na efflux among internodal cells was 0.2. No estimation of the variation of K efflux was possible; owing to the small amount of K^{42} coming out of the cells it was necessary to pool the efflux solutions from several cells and evaporate most of the water in order to have a suitably small sample for radioassay.

RESULTS

Na Fluxes with High External NaCl

Fig. 1 shows the time dependence of the Na fluxes of cells transferred from the 2 mM Na solution (control) to the 40 mM Na solution; the latter contains the highest concentration of NaCl used in this work. The decrease in Na influx with time is not associated with a comparable change in the electrochemical gradient. The membrane potential appears to assume a value of about −118 mv as soon as the cells are placed in the 40 mM Na solution, and the potential remains constant for at least 20 days (see Tables II and III below,

Experiments 31 and 50; the mean potentials for the control cells are −141 and −128 mv, respectively).

Fig. 1 also indicates that Na influx during the first week or so bears little relation to the condition of the cells so long as they remain alive. The phrase, "cells in poor condition," refers to cells which died within a few days after the final flux measurements were made. Since the same relative mortality

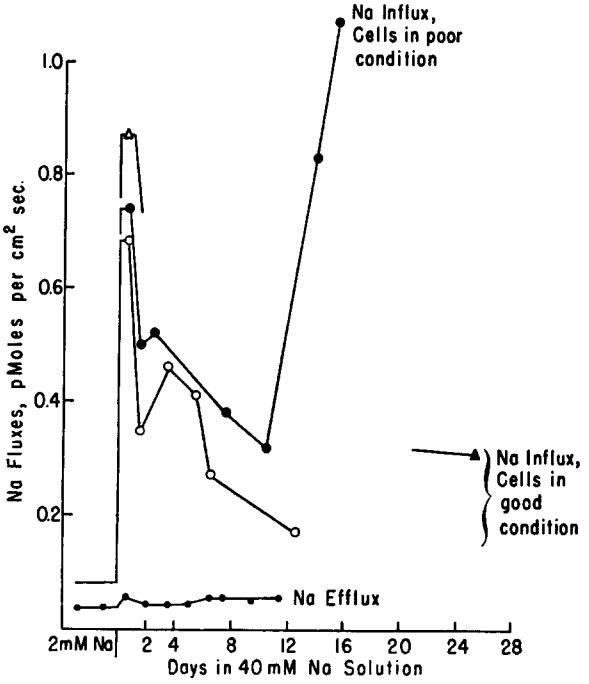

FIGURE 1. Unidirectional Na fluxes of internodal cells of *Nitella clavata* transferred from the 2 mM Na solution (control) to the 40 mM Na solution. For influxes each point is the mean for about 9 cells; the standard error of the mean is about 15 per cent in each case. For effluxes the same 5 cells were used throughout the experiment; each point is the mean for the 5 cells, with an SEM of about 9 per cent.

was observed in control cells (in 2 mM Na solution), the cause of death cannot be attributed to the 40 mM Na treatment. The very high fluxes observed in the final two measurements most probably represent one aspect of the death process. As Fig. 1 indicates the earlier flux measurements gave no indication that these cells were in some way different from long living cells, nor was their appearance noticeably different.

Sodium efflux is low and is affected only slightly by the change to a high NaCl solution. It appears to be roughly proportional to the vacuolar Na concentration, except for the first day of the altered conditions (Table II). In another experiment with the 2 mM Na solution the mean efflux for 17 cells

was 0.033 pmole/cm²sec. after a 2 day rinse; the vacuolar Na concentration was 22 ± 1 mM.

One is able to predict from Fig. 1 that the high Na influx and low efflux will lead to a rise in the vacuolar Na concentration, at first rapidly and then more slowly. This is borne out in Fig. 2. If net Na fluxes are calculated using

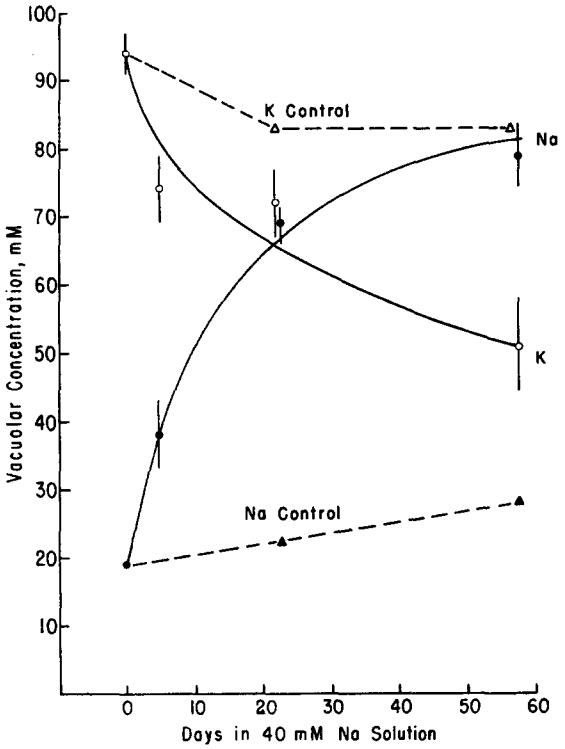

FIGURE 2. Vacuolar Na and K concentrations of internodal cells of *Nitella clavata* transferred to the 40 mM Na solution after 13 days of pretreatment in the 2 mM Na solution. The control values are for cells kept in the 2 mM Na solution. Each point is the mean for 4 to 6 cells; variation is expressed as the standard error of the mean. The K concentration of the external solution is 0.1 mM in both cases.

the mean diameter (650 μ) of these cylindrical cells, the values obtained for the 1st, 20th, and 40th days are approximately 1.0, 0.23, and 0.1 pmole/cm²-sec., respectively. Although agreement with the Fig. 1 results is not so good as one would hope, the predicted decrease in net Na influx with time is clearly manifested. The variation among cells and the smallness of the samples can readily account for the lack of good quantitative agreement here.

Cell death, just as with cells in poor condition, was not accelerated by the high NaCl treatment: of 8 test cells, 5 survived the 58 day treatment, whereas 4 of 13 control cells survived.

The gradual increase in the Na concentration of the control cells (Fig. 2) is likewise predicted by the difference between influx and efflux as given in Fig. 1. The mean net Na influx over the 58 day period was 0.03 pmole/cm²sec., coming close to the value obtained by algebraic addition of influx and efflux; *viz.*, about 0.04.

The results above show only that Na influx is high at the beginning of the 40 mM Na treatment and decreases with time, and that K is lost by the cells.

TABLE I

NET INCREASES IN THE NaCl CONCENTRATION OF *Nitella* CELLS IN THE 40 mM Na SOLUTION AFTER TRANSFER FROM THE 2 mM Na SOLUTION

Experiment No.	Time in 40 mM Na solution	Ion	Ion concentration		Δ Concentration	
			Initial	Final		
	days		*mM*		*mM*	
31	16	Na	30±1	53±3	+23	+13
		K	65±3	55±3	−10	
		Cl	98±5	116±5	+18	
13	33	Na	27±4	79±4	+52	+9
		K	79±6	36±6	−43	
		Cl	110±6	126±1	+16	
50	58	Na	19±1	79±5	+60	+17
		K	94±3	51±7	−43	
		Cl	129±6	139±8	+10	

Internodal cells transferred from the 2 mM Na to the 40 mM Na solution lose cellular K to the medium in exchange for Na. In addition to the equivalent exchange of these cations a net increase in the NaCl concentration of the vacuole occurs which appears to be completed within the first 16 days of the experimental treatment. The concentration increase is about 14 mM corresponding to a mean net NaCl influx of 0.21 pmole/cm² sec. if the influx period is taken to be the first 12 days of treatment. Each value is the mean for 4 to 10 cells ± standard error of the mean.

Along with these changes there occurs a net increase in the Cl concentration, as indicated in Table I. The net [Cl] gain of about 14 mM is apparently completed within 16 days; other flux data reported in this paper limit the minimum time required for its completion to about 12 days. The net Na influx occurring during this period may then be looked upon (with no implication concerning mechanism intended) as consisting of two fractions: (*a*) Na coming into the cell in exchange for K, and (*b*) Na balancing the net Cl influx. The latter fraction constitutes about one-half the net Na uptake if a 12 day period is assumed; the corresponding mean net Na and Cl influxes are each 0.21 pmole/cm²sec. Chloride flux data are lacking; such information is needed to determine how the eventual balancing of the Cl fluxes comes about. It

should be noted that although the osmolarity of the external solution is increased by 76 mM, that of the vacuolar sap increases by only 28 mM (if the osmotic change involves only Na, K, and Cl); this would result in a 20 per cent decrease in the hydrostatic pressure within the cell.

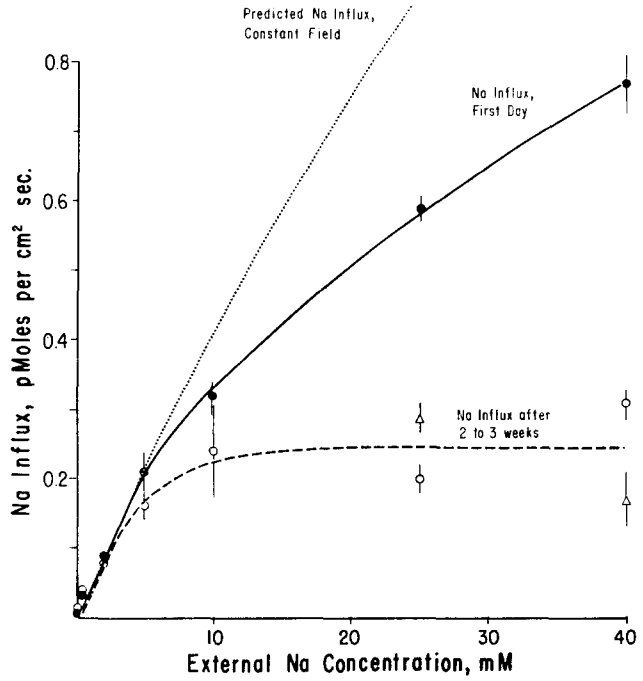

FIGURE 3. Dependence of unidirectional Na influxes on the external Na concentration during the 1st day of a given treatment and after 2 to 3 weeks of the same treatment. All cells were pretreated at least 10 days in the control solution (2 mM Na). The curve for the predicted Na influx is based on the assumption of a constant electric field across the plasmalemma, with the same Na permeability for all groups of cells; see equation (2). In these calculations a 13 mv decrease in potential for each tenfold increase in external Na concentration was used. Each point is the mean for about 8 cells, except for the 1st day 40 mM Na treatment in which 43 cells were used; variation is expressed as the standard error of the mean. The open triangles correspond to 2 weeks and the open circles 3 weeks of a given treatment.

Fig. 3 shows the concentration dependence of Na influx and how this dependence varies with time. Na influx becomes independent of the external [Na] for the concentration range 10 to 40 mM after 2 weeks of a given treatment. The dotted line indicates the expected Na influx for cells all having the same Na permeability (for details see legend for Fig. 3). The deviation from this expected behavior increases with increasing external [Na]; even for the first-day influxes this is true although to a lesser extent than for the prolonged treatments.

Na Flux Ratios

The flux ratios presented in Table II are those predicted for passive, independent Na movement as calculated by the equation below (12, 13); these are compared with the experimental ratios.

TABLE II

Na FLUXES, MEMBRANE POTENTIALS, AND VACUOLAR ION CONCENTRATIONS IN *Nitella clavata*

Experiment No.	Experimental solution* Measurement period	Mean vacuolar ion concentration (mid-period)			Na fluxes		Flux ratio		
		Na	K	E_m	In	Out	Experimental	Predicted	P_{Na}‡
	days	*mM*		*mv*	*pmole/cm²sec.*				*cm/sec.* × 10⁹
31	40 mM Na (0–0.1)	30	65	−120±7					
	40 mM Na (4–11)	42	59	−115±7					
	40 mM Na (13–20)	52	54	−118±6					
31 control	2 mM Na (0–20)	33	62	−141±3					
46	0.1 mM Na (0–0.1)	—	—	−147±5					
46 control	2 mM Na (0–0.1)			−138±6					
19, 47, 58	40 mM Na (0–1)	(30)	(76)	(−120)	0.77±0.04	(0.056)	14	147	4.1
13, 47	40 mM Na (12–25)	(52)	(54)	(−118)	0.24±0.03	(0.048)	5.0	77	1.3
13, 19	2 mM Na	30	76	(−140)	0.084±0.005	(0.036)	2.3	15.6	7.6
23	0.1 mM Na (0–1)	(40)	(57)	(−147)	0.005±0.001				8.6
23B	0.1 mM Na (21–22)	40	57	(−147)	0.004±0.001				6.9
34	40 mM Na (0–1)	35				0.056			
	40 mM Na (0–12)	49				0.048			
	2 mM Na (−12–0)	35				0.036			

Variation expressed as standard error of the mean. Four to 9 cells were used for potential measurements and 10 to 43 for influxes. The mean efflux values listed are for 5 cells; the SEM was 9 per cent of the mean. Vacuolar ion concentrations are means for 5 or 6 cells, with the SEM ranging between 5 and 10 per cent.

Values in parentheses have been obtained from other places in the table.

* Day zero is the starting point of the experimental treatment following pretreatment in the 2 mM Na solution.

‡ Constant electric field assumed.

$$\phi_i/\phi_o = [\mathrm{Na}]_o e^{-zEF/RT}/[\mathrm{Na}]_i \qquad (1)$$

The symbols are ϕ_i for unidirectional influx, $[\mathrm{Na}]_o$ for the concentration of Na outside the cell, $[\mathrm{Na}]_i$ for the vacuolar Na concentration, E for the potential difference between the external solution and the vacuole, and the others have their usual meanings. In order to make these calculations it was necessary to use information from several experiments conducted under the same conditions, as indicated by placing the "transferred" data in parentheses.

The experimental Na influx/efflux ratios are in all cases much smaller than those predicted by the equation; this is consistent with the hypothesis that a Na extrusion pump is present. Even with a nodal cell correction factor of 1.5 applied to the experimental ratio (see Methods), the results are essentially the same.

The Na permeability coefficients listed in Table II have been calculated in the usual way; *i.e.*, based on the assumption of a constant electric field across the plasmalemma (14, 15). The derived expression for influx is given below.

$$\phi_{NA_i} = -zEFP_{NA}[Na]_o/RT(1 - e^{zEF/RT}) \quad (2)$$

It appears that P_{Na} is constant for the concentration range 0.1 to 2 mM Na, that it decreases about one-half when the cells are first placed in the 40 mM Na solution, and then decreases with time to about one-sixth the control value. Efflux-derived P_{Na} values have not been calculated since most of the Na efflux is presumably pump-mediated.

K Fluxes

The potassium results represented in Table III show that the K influxes for the control cells are 2 to 3 times greater than those predicted for passive, independent movement. The K efflux value used in the calculation of the flux ratio is the actual experimental value obtained and so includes K effluxing from nodal cells, as well as the K from the internodal cells themselves. The measured efflux is consequently higher than the true value (see Methods for additional information). Whatever may be the extent of nodal cell contribution to the total K efflux, its effect is to partially hide the true disparity between the predicted and experimental flux ratios. Taken as they are, the data indicate that this disparity is appreciable, and thus the results are not consistent with the hypothesis that K movement is entirely passive and independent.

Raising $[Na]_o$ from 2 to 40 mM reduces K influx 20 to 60 per cent (Table III, Experiments 50 and 56), with the resulting K flux ratio being approximately that expected for independent movement. However, the efflux contribution of nodal cells must again be considered, and the large variation among the measured influxes should be noted. Lowering $[Na]_o$ from 2 to 0.1 mM results in a K influx increase of about 50 per cent. In this case the deviation of the experimental from the predicted flux ratio is in the same direction as that for the control solution, but of smaller magnitude. The same argument regarding the nodal cell efflux is applicable here.

Although in each of the above cases the change in membrane potential occurring with the change in solution composition is in the proper direction, *i.e.* in order to account qualitatively for the change in influx, these potential

changes are not large enough to be in agreement with the constant field assumption. This is better seen by making a comparison of the influx-derived P_K values, computed by an expression of the same form as equation (2). If this equation is adhered to, a part of the K influx change must be attributed to the change in potential, the other part to a change in P_K as shown in the

TABLE III

K FLUXES, MEMBRANE POTENTIALS, AND VACUOLAR IONS CONCENTRATIONS IN *Nitella clavata*

Experiment No.	Experimental solution* Measurement period	Mean vacuolar ion concentration (mid-period)		E_m	K fluxes		Flux ratio		P_K‡
		Na	K		In	Out	Experimental	Predicted	
	days	*mM*		*mv*	*pmole/cm²sec.*				*cm/sec.* × 10⁸
50	40 mM Na (0–0.1)	20	92	−118±5					
50 control	2 mM Na (0–0.1)	20	92	−128±4					
56	40 mM Na (0–1)			(−120)	0.11±0.03				230
56 control	2 mM Na (0–1)			(−140)	0.22±0.04				380
50	40 mM Na (13–14)	56	71	(−118)	0.05±0.01	(0.70)	0.07	0.14	110
	40 mM Na (16–17)	60	69	(−118)		0.70			220
	40 mM Na (21–24)	67	65	(−118)	0.12±0.02	(0.70)	0.17	0.15	260
50 control	2 mM Na (13–14)	21	87	(−128)	0.13±0.01	(0.34)	0.38	0.16	270
	2 mM Na (16–17)	22	86	(−128)		0.34			120
	2 mM Na (22–24)	22	83	(−128)	0.15±0.02	(0.34)	0.44	0.16	320
50	0.1 mM Na (13–14)	19	88	(−147)	0.24±0.02	(0.32)	0.75	0.36	420
	0.1 mM Na (16–17)	19	84	(−147)		0.32			210
	0.1 mM Na (21–24)	19	80	(−147)	0.17±0.02	(0.32)	0.53	0.40	280

Variation expressed as standard error of the mean. Six cells were used for the potential measurements and 8 to 16 cells for the flux measurements. Ion concentrations are from Fig. 2. Values in parentheses have been obtained from other places in Table III or from Table II.

* Day zero is the starting point of the experimental treatment following pretreatment in the 2 mM Na solution.

‡ Constant electric field assumed.

table. Thus, the apparent effect of raising $[Na]_o$ is to depress the K permeability; the depression of P_K appears to occur at the start of the 40 mM Na treatment and does not change with time. On the other hand, the P_K values computed from effluxes by equation (3) below (14, 15)

$$\phi_{K_o} = zFEP_K[K]_i e^{zFE/RT}/RT(1 - e^{zFE/RT}) \qquad (3)$$

would indicate that the raising of $[Na]_o$ does not affect the permeability for K leaving the cell (or perhaps causes it to increase slightly). This inconsistency, as well as the failure of the fluxes to conform to the values predicted

for the constant field model with independent movement of K ions, will be taken up in the Discussion. The fragmentary results presented below also have some bearing upon the adequacy of this model.

FRAGMENTARY RESULTS

1. The response of the membrane potential of *Nitella* to changes in external K was not immediate with the type of solution used in this work: when the 2 mM Na solution was gradually replaced by 2 mM Na solution plus 7.5 mM K_2SO_4, there was at first no change in potential. However, after 1 to 2 hours the potential started to decrease slowly, eventually stabilizing after 9 hours at about −20 mv, which is somewhat below the K equilibrium potential. Repolarization of the membrane is rapid when the original low K conditions are restored.

2. Complete replacement of the chloride in the 2 mM Na solution with sulfate resulted in a mean decrease in membrane potential of 13 mv. The rate of change of potential was rapid, corresponding to the change in solution composition.

3. The addition of 1 mM nitrate to a solution very similar to the 2 mM Na solution resulted in K flux behavior much different from that observed with the 2 mM Na solution. With $[K]_o$ at 0.22 mM the mean K influx was 0.56 ± 0.03 and the efflux 0.42 pmole/cm²sec. The membrane potential for another batch of cells under these same conditions was −104 mv (11). Using this value, the K influx/efflux ratio predicted for independent movement is 0.16 whereas the actual ratio is 1.3 (even without a correction for the nodal cells). Although this work was carried out on the California variety of *Nitella clavata*, not XO33, a similar result would be expected for XO33 since the two varieties behave quite similarly in many respects. A replication of chloroplasts under the above conditions occurs in both, the rate of the increase in chloroplast number being about 0.5 per cent per day. The "extra" chloroplasts are located in the vacuole. From this it appears that net K influx is associated with the net synthesis of cellular material.

DISCUSSION

Assumptions and Frame of Reference

The assumption that the plasmalemma is the principal barrier to cation movement has been made either implicitly or explicitly throughout this paper; the tonoplast is considered to be much more permeable to cations. Some justification for this is offered by MacRobbie's (6) study of the kinetics of Na and K movement in *Nitella flexilis*. Findlay and Hope (16) found the electrical resistance of the plasmalemma in *Chara australis* to be about 10 times that of the tonoplast. In itself this result is consistent with the above, but the absolute values of the resistances are much too low; *i.e.*, in compari-

son with resistances predicted from passive flux data. Whatever may be the significance of this, their other finding (16), that the protoplasm is approximately 10 to 15 mv more negative than the vacuole, is consistent with a higher protoplasmic cation concentration (6) and virtual equilibria of K and Na across the tonoplast. It appears probable that a very similar situation exists for *Nitella clavata* and, if so, the permeability coefficients calculated by equations (2) and (3) would be no more than 10 per cent in error.

A much more questionable assumption is that of independent ion movement. For independence of ion movement it is necessary to assume either that the permeation areas in the cell membrane are much larger than the ions or that the mobile ions in the membrane are so well separated that they do not interact with one another. The former assumption is ruled out because of the very effective discrimination between Na and K movements as found in these and most other cells. The latter assumption is acceptable in principle, at least under certain conditions. The procedure to be followed below is (*a*) to make an attempt at a model which can account for the magnitudes of the experimental flux ratios entirely on a "passive" basis in terms of the interdependence of ion movement (both Na and K net fluxes are "downhill") and (*b*) because of the failure of (*a*), to explain the results in the simplest way while still adhering as much as possible to the model; in this, all unexplainable ion movements will be categorized as "active" transport.

Models for the Interdependence of Ion Movement

Two models, both in essence dealing with single-file diffusion, are to be taken as the starting point for the present discussion. These are Mullins' (9) model in which successful ion movement into a membrane pore requires a good fit between the hydrated ion and the pore, and Rickert's (10) model for single-file diffusion. The latter involves the independent movement of a "hole" (*Leerstelle*), the remaining sites in the pore being satisfied by mobile ions. This treatment appears to be more satisfactory than the "billiard ball" approach of Hodgkin and Keynes (17). An important result of Rickert's treatment is that such pores will sometimes be one way pores, *i.e.* the successful transfer of a tracer ion across the membrane will occur only in one direction, the only conditions being a very small electrochemical gradient, and, on the average, no more than one hole at a time in a pore with several sites. The process for Na influx, for example, would be seen as one Na ion moving from the pore into the cell, leaving a hole at the end of the pore, with the hole then migrating through the pore in one site jumps until reaching the outside. Mullins' model complements this by showing that the specificity of the partitioning of ions between the solution and membrane phases depends on the closeness of fit between ion and pore. In both models saturation effects and competition are possible. In the above example virtual saturation of the pore

occurs; in the case of a pore with a single ion present at one time, complete independence of movement is had in one dimension.

It seems to the writer that a much more workable model for passive ion movement is possible if the permeation areas are considered to be slits or clefts rather than cylindrical pores. Such "slit-pores" would permit within them movement in two dimensions in contrast to the one dimension required in single-file diffusion. A slit-pore, because of its narrowness, could still exclude the larger, hydrated Na ions while permitting K movement in both directions across the membrane. Moreover, for a situation sufficiently removed from equilibrium a magnification of the flux ratio in favor of the net flux direction would be expected because of an increasingly more effective coupling of ion movements with increasing net flux.

Unsuccessful Application of the Model to the Data As a Passive Explanation

From Tables II and III it is apparent that Na efflux and K influx are both too high, *i.e.* relative to Na influx and K efflux, respectively, if independence of movement is assumed. The only possible way to account for this by the model above is to suppose (*a*) that the K slit-pores are one way outward pores and that all of the Na efflux (0.04 pmole/cm^{2}sec.) is carried along by the over-all or "hybrid" electrochemical gradient (10) in these pores, and (*b*) that the Na slit-pores are one way inward pores through which some K also moves, likewise along a hybrid electrochemical gradient (10). Possibility (*a*) is conceivable on the basis of the electrochemical gradient which favors K efflux, but it seems very questionable on other grounds. The partitioning of K and Na into these pores from inside the cell would so strongly favor K (because of its high concentration and better fit in the pore) that only a negligible amount of Na could be transferred out of the cell in this way. Possibility (*b*) must also be ruled out because the hybrid electrochemical gradient in the Na pores would favor efflux rather than influx if all of the K influx occurs through these pores.

Interpretation of the Na and K Fluxes

Since the model proposed above completely fails to account for the observed K and Na fluxes, it is necessary to assume that Na is actively extruded and that most of K influx under the control conditions is active. The net influx of K up a steep electrochemical gradient when nitrate is present offers some basis for the latter supposition. As noted under Methods the K effluxes reported for the three conditions probably are about twice the true values; the cell sap analyses in Table I and other analyses not reported here tend to bear this out. Any departures of the passive fluxes from the behavior predicted by equations (1) to (3) are then to be interpreted as being due to

the interdependence of movement of the inwardly and outwardly moving ions through the slit-pores. The slit-pore structure is still adhered to since it is necessary to account in some way for the cell's capacity to discriminate between Na and K. The summarized results are presented in Table IV; representative values have been chosen and the K effluxes have been adjusted as indicated in the discussion below.

1. NA INFLUX

Because the P_{Na} values for both the 0.1 and 2 mM Na solutions are about the same, it appears that saturation of the Na slit-pores does not occur unless

TABLE IV
SUMMARY OF EXPERIMENTAL RESULTS

Experimental solution*	Ion concentration			E_M	Fluxes			
	Na	K	Cl		Na in	Na out	K in	K out
	mM			*mv*	*pmole/cm²sec.*			
0.1 mM Na	28	76	110	−147	0.004	—	0.2	0.2
2 mM Na				−136	0.084	0.04	0.14	0.18
40 mM Na, 1st day				−120	0.77	0.06	0.1	—
40 mM Na, 2–3 wks.	57	61	124	−118	0.3	0.05	0.1	0.35

* The K concentration in the medium is 0.1 mM; the Cl concentration is equal to that of Na plus 1 mM.

$[Na]_o$ is very high. Even for the 40 mM Na solution only partial saturation seems to occur. Although the initial influx with $[Na]_o$ at 40 mM is only about half that predicted by equation (2), it clearly is not a saturation value; thus it is to be ascribed to partial saturation of the slit-pores (Fig. 3). The further decline of P_{Na} with time is more difficult to explain but appears to be associated with the increase in cellular [Na] and the concomitant decrease in [K] (the membrane potential remains at about −118 mv during this time). The vacuolar K/Na concentration ratio decreases to about 40 per cent of its initial value, which correlates well with the decrease in Na influx, to about 40 per cent of its initial value. This suggests that Na tends to clog up its own pores as the cellular Na concentration builds up. One way of looking at this is to suppose that although these pores are essentially one way Na inward pores due to the electrochemical gradient, K ions do "reflux" into them from the inward side of the membrane and by their presence in sufficient number permit a much greater mobility of Na ions in the membrane. The loss of the dependence of Na influx on $[Na]_o$ (Fig. 3) is also consistent with this explanation.

2. Na EXTRUSION

Na extrusion appears to be proportional to the vacuolar [Na]. When this is 40 mM the extrusion rate is about 0.04 pmole/cm²sec. Under the control conditions this is one-half the magnitude of Na influx, and so the cellular [Na] slowly increases. This appears to be consistent with the course of events under natural conditions, as MacRobbie (6) has reported. Unfortunately no Na efflux measurements under the 0.1 mM Na condition were carried out; by such measurements exchange-diffusion (18) might have been detected. Since cell sap analyses on several batches of cells do not seem to indicate a decrease in cellular [Na] under low $[Na]_o$ conditions, it is possible that the Na efflux attributed here to active Na extrusion is actually due to exchange-diffusion.

3. K FLUXES

The effect of high $[Na]_o$ is to cause a decrease in K influx and that of low $[Na]_o$ is to cause an increase in K influx. To be consistent throughout, the interpretation to be applied here sets active K transport at about 0.1 pmole/cm²sec. Thus under the control conditions passive K influx is about 0.04 and efflux is about 0.2. This is in agreement with equation (1) for independent ion movement with a linear potential change across the membrane. For the 0.1 mM Na solution total K influx is about 0.2, making the passive influx about 0.1 pmole/cm²sec. K efflux can be taken as 0.2 since cells in this solution appear to have balanced K fluxes (cell sap analyses not reported). In this case fairly good agreement is had with the predicted flux ratio of 0.4. Thus under low $[Na]_o$ conditions the passive portion of K movement appears to be independent movement in a constant field.

For the 40 mM Na solution, where the measured K influx varies between 0.05 and 0.12 pmole/cm²sec. (Table III), passive K influx must be taken as zero if it is assumed that active K influx is 0.1 as it is under the other conditions. This means that K movement in the slit-pores is one way outwardly. K efflux approximately doubles under these conditions, a conclusion obtaining either from the primary data of Table III or from a resolution of the conflicting data of Tables I and III and Fig. 2; the values adopted as a result of this resolution are presented in Table IV, and if Fig. 2 is also to conform to this resolution, the vacuolar [K] curve must be drawn almost linearly. Although the conversion of a two way slit-pore with essentially independent K movement (control solution) to a one way pore would appear to be a rather sharp change for an 18 mv decrease in membrane potential, it is not inconcievable. The finding of Gaffey and Mullins (4) that no influx of tracer K occurs during an action potential in *Chara* indicates that the K pores are one way pores under those conditions.

Origin of the Membrane Potential

The insensitivity of the membrane potential to $[K]_o$ indicates that the potential is not a K diffusion potential under the usual conditions. It apparently may become a K diffusion potential under certain circumstances, namely high external [K]. Somewhat similar results reported for *Chara* by Hope and Walker (19) and Oda (20) suggest that either high $[K]_o$ or low $[Ca]_o$ is the condition required to permit K to move fast enough through the membrane to control the potential. Under normal conditions it appears that the potential may be determined by the electrogenic activity of the chloride pump; this is suggested but in no way proved by the lowering of the potential when external chloride is replaced by sulfate. The small magnitudes of the K and Na fluxes under the conditions of this work correspond to a specific membrane resistance of at least 1 megohm cm^2, which is sufficiently high to be consistent with the interpretation above.

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