Potassium Transference in *Nitella*

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ABSTRACT   Transmembrane movements of K^+ and Cl^- were studied under a variety of experimental conditions. Potassium was found to carry more than 50% of an externally applied inward positive current. The increase in K^+ influx was much greater than that predicted by the purely passive model. The increase in Cl^- efflux accounted for <10% of the applied current, in agreement with the value predicted for passive movement. 2,4-Dinitrophenol (DNP) caused an 80% reduction in K^+ transference and a corresponding increase in the measured electrical resistance of the membrane. DNP also reduced the isotopically measured resting K^+ influx and caused a substantial increase in both Cl^- influx and efflux. Lowering of the pH from 5.7 to 4.7 also reduced the net K^+ influx but without drastically altering the membrane resistance. It appears the major portion of an externally applied current does not travel through passive channels, but rather is shunted through a different membrane component. In conjunction with evidence previously establishing the H^+ pump as the primary ion pump in *Nitella*, the data presented here are consistent with a K^+/H^+ exchange mechanism which can account for the observed net K^+ accumulation and maintenance of the membrane potential above the electrochemical equilibrium potential of the major ions. This mechanism appears to be a likely candidate for the current shunt.

INTRODUCTION

This study is concerned with the relationship of K^+ fluxes to the electrically measured membrane conductance and the level of the resting potential of giant internodal cells of *Nitella*. In such studies it has been customary to assume that electrogenic ion pumping is present and that a relatively large passive return current serves to electrically balance it. The acceptability of this idea was greatly influenced by Kitasato's (1968) report suggesting the distinct possibility of H^+ as the major ion both in active extrusion and in the passive return current (cf. Anderson, 1973). Before this an unresolved problem existed in that the typical measured electrical conductance of Characean cells was more than 10 times greater than that predicted from the resting fluxes of K^+, Na^+, and Cl^- (Williams et al., 1964), as calculated by the Goldman (1943) flux equation. This problem was apparently solved by attributing the unexplained conductance to H^+ (Kitasato, 1968).

It is to be noted that membrane conductance is computed from the displacement of the resting potential when an applied current of known magnitude is

passed through the membrane. The explicit assumption here is that the current passes only through passive channels and that any active electrogenic mechanism acts as a constant current generator, remaining unaffected by this membrane perturbation. Spanswick's (1972) challenge to this assumption consisted essentially of the contention that the electrogenic pump is quite sensitive to imposed changes in the membrane potential, adjusting its rate in response to slight changes in the potential, i.e., pumping faster during a depolarization (inside less negative) and slower during a hyperpolarization. In terms of the H^+ hypothesis, the best test for this would be the lowering of the external pH when a depolarizing current is applied. This measurement has not yet been made.

All attempts to detect an external pH rise when a hyperpolarizing current was applied have failed (Anderson, 1973: pp. 137, 142, 236). Leaving aside the question of the H^+ pump, this result casts doubt on the importance of H^+ as a passive current carrier. Thus, the original puzzle of the large conductance has persisted. This has been complicated by difficulties in efforts to fully identify the ions that carry applied currents. Such efforts have apparently been limited to two studies (Walker and Hope, 1969; Williams et al., 1972): no more than about 40% of the applied current could, with certainty, be attributed collectively to K^+, Na^+, and Cl^-.

Our strategy in attacking this problem in the present work was to use a higher K^+ concentration in the medium (1.0 mM as compared with the usual 0.1) in the hope that the K^+ conductance would be enhanced, with K^+ then carrying most of the inward current during hyperpolarization. Earlier it had been found (Brown et al., 1973) that with this K^+ concentration, *Nitella* cells maintained an average resting potential of -152 mV, some 40 mV more electronegative than the K^+ equilibrium potential. This is significant in that it suggests a definite electrogenic component of the resting potential.

Potassium fluxes were measured with *Nitella* cells both at rest and during application of a hyperpolarizing current across the membrane at pH 5.7. Similar experiments were conducted on cells treated with 2,4-dinitrophenol (DNP). Chloride movement was also studied at rest and during current application. Resting fluxes were measured for both K^+ and Cl^- with and without DNP and at pH 4.7 to further characterize the ionic pathways. The membrane potentials and electrical resistances were recorded under all of these experimental conditions.

MATERIALS AND METHODS

Culturing of Nitella and Preconditioning of Cells

Plants of *Nitella clavata* were grown in open 3-1 cultures with aeration under 1,350 lx illumination provided by Sylvania Grolux (Sylvania Lighting Products, Hillsboro, N.H.) and cool-white fluorescent lamps. A 16-h light and 8-h dark cycle was used. The culture solution was similar to Forsberg's (1965) containing 4 mM Tris at pH 7.0. Because of the degradation of Tris, NH_4^+ present varied between 0.5 and 2.5 mM, averaging about 1 mM. Internodal cells ranging between 0.07 and 0.08 cm in diameter and between 2 and 3 cm in length were harvested and placed in a solution containing 1.0 mM KCl, 0.1 mM NaCl, 0.1 mM $CaCl_2$, and 0.1 mM $MgCl_2$ at pH 5.7 for 6-8 days before use. This solution

is designated as K solution throughout this work. During preconditioning, the external solution was changed every 2 days. The lighting during the preconditioning and experimental periods was 480 lx from cool-white fluorescent lamps. The temperature was 22°C.

Membrane Potentials and Areal Resistance

Membrane potentials were measured with Ag/AgCl glass microelectrodes as previously described (Rent et al., 1972). The electrical potentials reported here are actually those measured across the cell wall, plasmalemma, cytoplasm, and vacuolar membrane; conventionally, these are referred to as membrane potentials (as they are here), but the designation is somewhat inaccurate.

Membrane electrical resistances were measured by the method of Hogg et al. (1968) in which the leaky cable properties of the cell are corrected for. Just as in the E_m measurements, the reported resistance properly refers to that between medium and vacuole; however, it appears that the resistance is almost entirely attributable to the plasmalemma (Skierczynska et al., 1975).

Undirectional K^+ Fluxes

Potassium uptake was measured with ^{42}K at a maximum spec act of 13 Ci·mol^{-1}. After exposure to tracer, cells were given a three-phase, 2-h rinse in a large excess of nontracer solution. Radioactivity in a living cell was assayed by placing a cell in a specially designed holder directly beneath a Geiger-Müller tube. An appropriate correction curve for cell geometry was constructed by taking a number of cells and fragmenting and drying them to give the material the same geometry as that of samples of known radioactivity.

Potassium efflux was measured by collecting ^{42}K lost from a cell in 4.0 ml of nontracer solution. 50 µl of 1.0 M NaHCO$_3$ was added to 1.0 ml of sample which was then dried and counted in a Nuclear-Chicago low background beta counter (Nuclear-Chicago Corp., Des Plaines, Ill.). After ^{42}K uptake, rinse periods of several hours and two consecutive efflux periods were used to be certain that $^{42}K^+$ exchanging from the cell wall was not included in the efflux samples (Hope, 1963).

Potassium Uptake with Applied Hyperpolarizing Current

For measurement of ^{42}K uptake during the passage of an external hyperpolarizing current through the membrane a Geiger-Muller tube was mounted behind a thin plastic partition on a Plexiglass trough containing the cell. The tube axis was perpendicular to the axis of the cell. Current was delivered to the inside of the cell via microelectrode in the same manner used to measure membrane resistance. The resting ^{42}K influx was first established during one or two 30- or 60-min influx periods. After exposure to tracer solution, the cell and trough were rinsed repeatedly until no fluctuation in radioactivity was observed. This usually required 20-30 min during which the total volume of the trough was replaced 40 times. The cell was then hyperpolarized in the presence of tracer with a current of about 0.4 µA, rinsed and assayed. A final control was normally obtained following the applied current period. After these uptake experiments the electrodes were removed, and the cell was then fragmented, dried, and counted as described above. This permitted calculation of absolute flux values.

Chloride Fluxes at Rest and under Applied Current

Chloride uptake was measured using ^{36}Cl in K solution at 114 mCi·mol^{-1}. Unidirectional flux measurements were made in a manner identical to that outlined above for ^{42}K except that the rinse period was 1.5 h.

Efflux on a ^{36}Cl-loaded cell was measured with an applied hyperpolarizing current by sealing off the trough, applying the current, then removing external solution for radioassay.

Cellular Ion Concentrations

The internal K$^+$ concentration of cells used for ^{42}K efflux experiments was determined by breaking cells open in 4.0 ml distilled H$_2$O and measuring with a Corning cation electrode (no. 476220, Corning Glass Works, Corning, N.Y.). Corrections were made for Na$^+$ and NH$_4$$^+$ interference with the electrode.

Ammonia content of single cells was determined in a total volume of 10 ml by the Nessler method (Taras, 1958); 15 min after the addition of reagents, the absorbance was read at 450 nm. Turbidity in the reaction mixture was prevented by adding 0.1 ml of 4.4% (wt/wt) gum arabic solution before the addition of the Nessler's reagent. Vacuolar Cl$^-$ was determined by diluting collected sap 100-fold and measuring with a Cl$^-$-specific electrode.

RESULTS

Level of Membrane Potential

A mean membrane potential of -152 ± 3 mV was obtained on 40 cells in K solution. This is 40 mV more negative than the estimated E_K of -112 mV based on a cellular [K$^+$] of 80 mM.

When [K$^+$]$_0$ was decreased from 1.0 to 0.1 mM while [Na$^+$]$_0$ was simultaneously increased from 0.1 to 1.0 mM, very little change in E_m was observed. Others have previously noted the insensitivity of the membrane potential of Characean cells to changes in external [K$^+$] when Ca^{++} is also present in the medium (e.g., Hope and Walker, 1961). The basis for this insensitivity is not clear as Hope (1963) has shown that the magnitude of the K$^+$ influx strongly reflects the external K$^+$ concentration. These results lead to the conclusion that transmembrane K$^+$ movements, whatever the mechanism or mechanisms involved, do not contribute in a simple way to the determination of the E_m level. Rigorous evidence for a passive component of K$^+$ influx in Characean cells is lacking (Hope and Walker, 1975).

Dependence of K$^+$ Fluxes on External [K$^+$]

Fig. 1 indicates that the unidirectional K$^+$ influx is linearly dependent on external [K$^+$] within the range of concentrations used. It is to be noted that [Cl$^-$]$_0$ was also varied with [K$^+$]$_0$. The overall mean unidirectional influx for the control concentration of 1.0 mM K$^+$ was 1.73 pmol·cm^2·s^{-1} whereas the efflux was 0.14. Thus, the mean net influx was 1.6 pmol·cm^{-2}·s^{-1}.

Actual measurement of total vacuolar K$^+$ from 0 to 11 days showed an increase in internal K$^+$ from 20 mM at day 0 to 100 mM at day 11. The average internal K$^+$ concentration of 6- to 8-day cells used in this work was 80 mM. For the 11-day period the mean net influx was about 1.5 pmol·cm^{-2}·s^{-1}, in reasonable agreement with the isotopically measured fluxes of 7-day cells. The net K$^+$ uptake was approximately balanced by the decrease in the NH$_4$$^+$ content of the cells. It appears that K$^+$ is not exchanged for NH$_4$$^+$ but for H$^+$ which is extruded as NH$_3$ leaves the cell passively, resulting in an internal acidification

(Barr et al., 1974). This conclusion derives from the fact that NH_3 permeability coefficient is the same for both inward and outward movement, 9×10^{-4} cm·s^{-1}. In addition, recent evidence has been obtained for an H^+/K^+ exchange in the absence of NH_3 movement (Barr et al., 1977). The cellular Cl^- and Na^+ concentrations were essentially constant at 87 and 14 mM, respectively; during the 11 days after harvest, deviations from these values were by no more than 2 mM.

If the assumption is made that K^+ efflux is entirely passive and independent, then a calculation of the fraction of the K^+ influx that is passive can be made (Goldman, 1943). Based on a $[K^+]$ of 80 mM and an E_m of -152 mV, the potassium permeability coefficient (P_K) is 1.1×10^{-7} cm·s^{-1} and the passive K^+
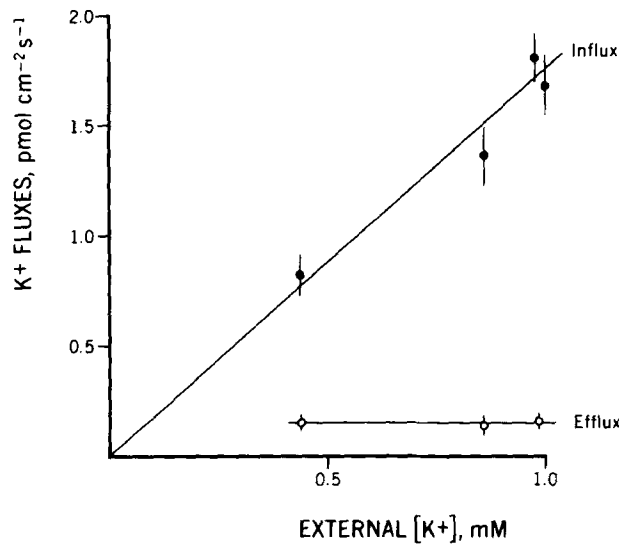

FIGURE 1. Dependence of unidirectional K^+ fluxes of *N. clavata* on the external K^+ concentration. 10, 32, 23, and 40 cells were used, respectively, for the 0.44, 0.86, 0.98, and 1.00 mM concentrations. Variation is expressed as the standard error. The solution containing 1.00 mM K^+ is the K solution; where the K^+ concentration is less, the Cl^- concentration has been reduced by the same amount. The cells were 6-8 days old.

influx is 0.66 pmol·cm^{-2}·s^{-1} or 38% of the total influx. There is, however, no compelling basis for adhering to this model.

Potassium and Cl^- Transference Numbers

When external hyperpolarizing currents of 0.4-1.0 μA·cm^{-2} were applied to *Nitella* cells, most of the current was carried by K^+, as shown in Table I. The K^+ current density is defined as the net K^+ flux, and the transference number (T_K) is defined as the fraction of the applied current carried by K^+. Thus, the T_K averaged 0.83 for cells in solutions containing 0.86-1.00 mM K^+. The mean value for Cl^{-1} was 0.07 as determined in 16 measurements by the increase in Cl^- efflux

during hyperpolarization. Figs. 2 and 3 show details for typical cells on which transference was measured. According to the definition, the mean T_K is 0.83, with 0.57 of this corresponding to the increase in net K^+ influx. Along with the T_{Cl} of 0.07, 90% of the applied current can be formally accounted for. However, there remains the question of how the net K^+ influx at rest is balanced and how this balancing flux is affected by the hyperpolarization.

None of the applied current was carried by H^+. In three attempts to demonstrate an external pH increase due to an inward H^+ current during current applications, no significant pH changes were observed. If H^+ had

TABLE I

EFFECT OF AN APPLIED HYPERPOLARIZING CURRENT ON THE K^+ INFLUX
OF INDIVIDUAL *NITELLA* CELLS

Exp.	$[K^+]_o$	Membrane conductance	K^+ influx resting during current		Applied current density	Transference no.*
	mM	*µho·cm⁻²*	*pmol·cm⁻²·s⁻¹*		*peq· cm⁻²·s⁻¹*	
33	0.44	61	0.90	4.55	7.64	0.58 (0.48)
34	0.44	77	1.04	3.60	6.94	0.50 (0.38)
48	0.86	13‡	2.86	4.85	4.40	1.07 (0.45)
42	0.98	28	1.07	3.56	6.70	0.51 (0.37)
44	0.98	25	0.90	3.05	5.00	0.58 (0.43)
61	1.00	31	2.28	6.85	7.10	0.95 (0.65)
62	1.00	30	2.88	8.53	9.33	0.90 (0.60)
63	1.00	35	1.66	8.50	9.00	0.93 (0.76)
64	1.00	34	1.66	8.15	9.42	0.85 (0.69)
Mean values excluding Exp. 33, 34		28±3	1.90±0.30			0.83 (0.57)

The resting influxes are mean values obtained from two or three 1.0-h measurement periods either before or before and after the application of current for 1.0 h. In experiments 63 and 64, 0.5-h periods were used.

* To calculate transference numbers, an efflux of 0.14 pmol·cm⁻²·s⁻¹ was assumed, equal to the mean resting efflux. The values in parentheses represent transference attributable to the increase in the net K^+ flux during current application.

‡ This cell had an unusually low resting potential of −113 mV and was hyperpolarized to −163 mV by the applied current. The mean E_m of the other cells was −154 mV, with the mean hyperpolarization being 19 mV.

carried 100% of the applied current, the external pH would have risen from pH 6 to pH 7 in 3 min under our conditions.

In Fig. 4 a correlation between the T_K and the K^+ resting influx is apparent: there is a monotonic rise in T_K with increasing K^+ influx up to about 2 pmol·cm⁻²·s⁻¹ at which value the T_K approaches unity. Of the four points that have T_K's of about 0.5, two of them correspond to low K^+ concentrations (0.44 mM) whereas the other two are for low influxes not specifically ascribable to any known factor. These results seem to indicate that the fraction of applied current carried by K^+ depends upon both the availability of K^+ in the medium and the inward K^+ transport capacity of the membrane, whatever its nature may be.

This relationship holds even when DNP is present (details below). Also, when the product of these two factors is sufficiently large, K^+ will predominate as the current carrier, with T_K approaching unity. The curve is drawn through the origin because in the special case of zero $[K^+]_0$, the K^+ current will be zero or very nearly zero.

Comparison of Calculated and Measured Conductances

As indicated under the flux results, the passive component of K^+ influx may be said to account for 38% of the total K^+ influx if the conventional "active/passive"
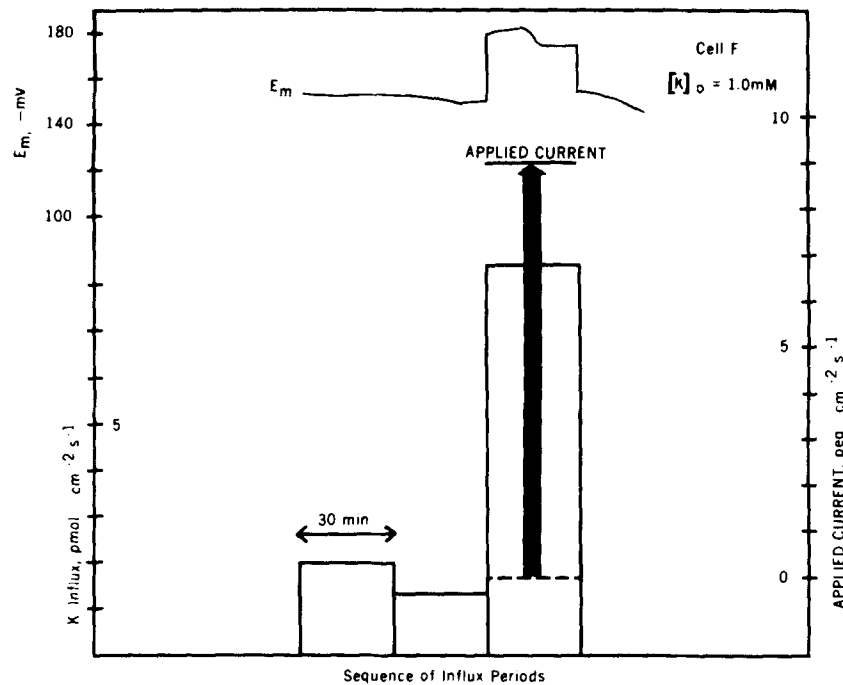

FIGURE 2.   Potassium influx of a 9-day-old cell of *N. clavata* during application of a hyperpolarizing current as compared to the influx at rest. The plain bars represent influx measurements at rest. The bar with the vertical arrow represents the influx during the application of a current of 9.0 peq·cm^{-2}·s^{-1}, causing a mean hyperpolarization of 25 mV. This cell is identified as experiment 63 in Table I.

assumptions are made. If so, the net passive K^+ influx of 0.52 pmol·cm^{-2}·s^{-1} corresponds to a resting K^+ conductance of 1.3 μmho/cm^2 as calculated by the following formula:

$G_K = F \bar{J}_K/E_K - E_m$, where G_K = resting K^+ conductance, $\bar{J}_K$ = net K^+ flux, and F = Faraday constant. As can be seen in Table I the measured membrane conductance of cells subjected to prolonged hyperpolarizations was 28 μmho·cm^{-2} on the average, with the K^+ conductance accounting for most of it, 23 μmho·cm^{-2}. There is almost a 20-fold discrepancy here. Even if the entire net K^+ influx of 1.6 pmol·cm^{-2}·s^{-1} is used to calculate the resting conductance,

a value of only 3.8 μmho·cm^{-2} is obtained, about one-sixth the measured value. Thus, neither the formula above nor the Goldman (1943) equation is applicable to K$^+$ movement when a net current is drawn through the membrane via an external circuit.

It is to be noted that the membrane conductances obtained from short pulses of a few seconds were typically about 60 μmho·cm^{-2}, about twice the value obtained for prolonged hyperpolarizations. Walker and Hope (1969) observed similar behavior in *Chara corallina*.
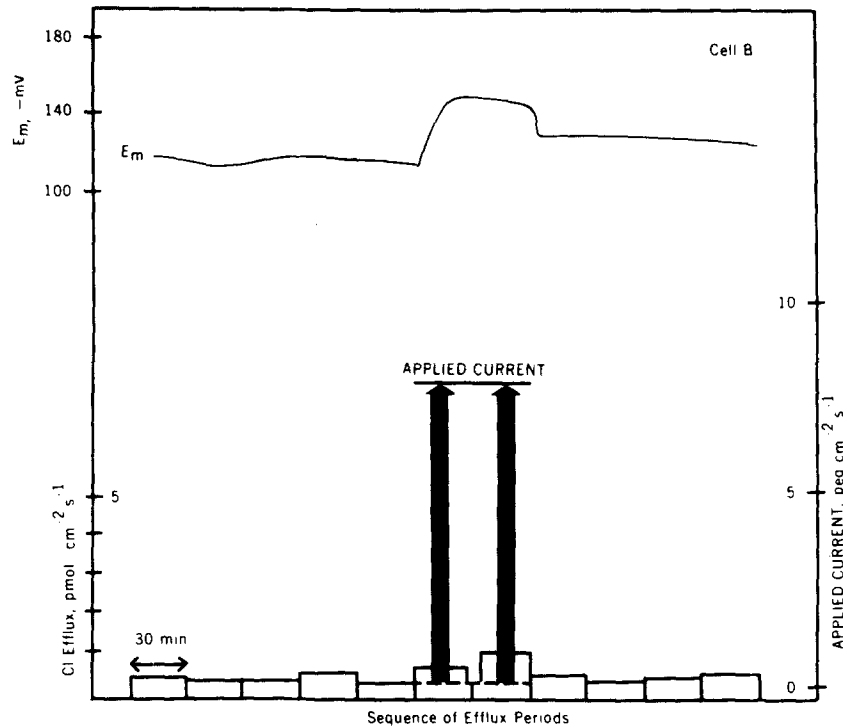

FIGURE 3. Chloride efflux of a typical internodal cell of *N. clavata* during application of a hyperpolarizing current as compared to the efflux at rest. The plain bars represent efflux measurements at rest. The two bars with vertical arrows represent efflux measurements during the application of a current of 7.7 peq·cm^{-2}·s^{-1}, causing a mean hyperpolarization of 26 mV. The cell was 6 days old.

We have observed very little, if any, rectification under these conditions; this perhaps is due to the relatively high external [K$^+$] present. Spanswick (1973) found the membrane of *Nitella translucens* to exhibit a perfectly ohmic behavior over an extended range in the light, but not in the dark.

Effects of DNP on Membrane Properties

In Table II are presented the results of experiments in which DNP was used as a metabolic poison, in K solution at pH 5.7. The assumed action of DNP is to

depress the energy supply and consequently eliminate much of the active transport. In two experiments the resting K^+ influxes were reduced by 89 and 79%. In another DNP experiment involving three cells, the T_K's were reduced by 94, 88, and 65% based on the mean control T_K of 0.83. The similarity in the degree of inhibition suggests the possibility of a common mechanism for the two modes of transport, or, at least, a common rate-limiting step. This is shown more clearly in Fig. 4 where the mean T_K value for the DNP cells is seen to conform quite well to the general relationship found between the T_K and the resting K^+ influx.

Two other major effects of DNP observed were a mean depolarization of the membrane to -104 mV and a large decrease in electrical conductance, corre-
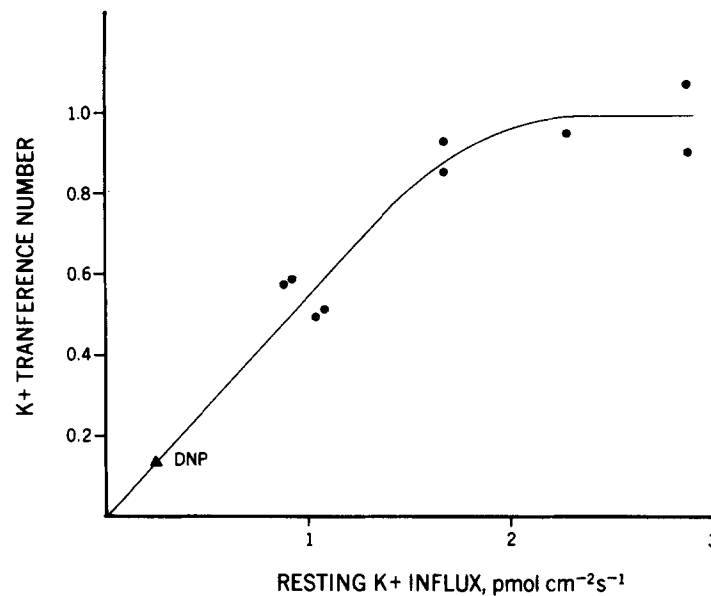

Figure 4. Relationship between the K^+ transference number and the resting K^+ influx in *N. clavata*. The values were obtained from Table I. The point for DNP-poisoned cells was obtained from the mean values presented in Table II.

sponding to the decrease in T_K (Table II). Because -104 mV is slightly more positive than E_K, a small net efflux of K^+ is predicted if the movement is passive; Table II indicates a net efflux of 0.3-0.4 $pmol \cdot cm^{-2} \cdot s^{-1}$. Further evidence for the passive behavior of DNP-treated cells was obtained by increasing the external $[K^+]$ to 10 mM (Table III): depolarization to -55 mV was observed, close to the calculated E_K value of -52 mV. Deviations from the theoretical value may be due to an increase in P_{Cl} (see below). Fig. 5 depicts the time-course of the effects of DNP on the membrane potential and resistance.

Table II lists a mean conductance of 8 $\mu mho \cdot cm^{-2}$ for five cells treated with DNP. The three (different) cells used for transference measurements had conductances of 3, 4, and 14 $\mu mho \cdot cm^{-2}$, respectively, not significantly differ-

ent from the above. In the transference experiment the low conductances, in combination with the applied currents used, resulted in hyperpolarizations of about 70 mV, i.e., from about -100 to -170 mV. Thus, the E_m levels in force during transference measurements were roughly the same as those for the controls.

TABLE II

EFFECTS OF DNP AND LOW pH ON *NITELLA* MEMBRANE PROPERTIES

Membrane property	Units	50 μM DNP in K solution	Control K solution pH 5.7	K solution pH 4.7
Resting potential	mV	-104 ± 7*	$-152\pm3\ddagger$	$(-89)\S$
Electrical conductance	μmho·cm^{-2}		$59\pm7\parallel$	$(60)\S$
Electrical conductance	μmho·cm^{-2}	8 ± 2*	$28\pm3\P$	
K influx	pmol·cm^{-2}·s^{-1}	0.19 ± 0.01**	1.75 ± 0.10**	0.43 ± 0.01
K influx	pmol·cm^{-2}·s^{-1}	$0.35\pm0.01\ddagger\ddagger$	$1.63\pm0.16\ddagger\ddagger$	0.37 ± 0.04
K efflux	pmol·cm^{-2}·s^{-1}	0.55 ± 0.03**	0.27 ± 0.10**	0.47 ± 0.06
K efflux	pmol·cm^{-2}·s^{-1}	$-$	$0.14\pm0.02\ddagger$	
P_K, influx-derived	nm·s^{-1}	0.5**	$-$	1.2
P_K, influx-derived	nm·s^{-1}	$0.8\ddagger\ddagger$	$\ldots$	1.3
P_K, efflux-derived	nm·s^{-1}	1.0**	2.1**	0.52
P_K, efflux-derived	nm·s^{-1}	$-$	$1.1\ddagger$	
K conductance (resting, see text)	μmho·cm^{-2}	1.4**	0.9**	$-$
K conductance (resting, see text)	μmho·cm^{-2}	$\ldots$	$1.3\ddagger$	
K transference no. (applied current)	μmho·cm^{-2}	$0.07, 0.10, 0.29\S\S$	$0.83\P$	$-$
Cl influx	pmol·cm^{-2}·s^{-1}	$0.95\pm0.04\parallel\parallel$	$0.09\pm0.01\parallel\parallel$	$0.93\pm0.13\parallel\parallel$
Cl efflux	pmol·cm^{-2}·s^{-1}	$1.82\pm0.09\parallel\parallel$	$0.13\pm0.01\parallel\parallel$	$1.41\pm0.26\parallel\parallel$
P_{Cl}, efflux-derived	nm·s^{-1}	$0.05\parallel\parallel$	$0.003\parallel\parallel$	0.05
Cl conductance (resting)	μmho·cm^{-2}	$0.4\parallel\parallel$	$0.015\parallel\parallel$	$\ldots$

* Mean value for five cells, measured after 1- 2 h in DNP.

‡ Overall mean values, 30 cells.

§ Taken from Brown, et al. (1973).

∥ Mean value for 11 cells, short DC pulses.

¶ From Table I, hyperpolarizations for prolonged periods.

** Mean values on 7-10 cells. The experimental period was 3 h for the DNP-treated cells and 13-21 h for the controls.

‡‡ Same as but on a different batch of cells.

§§ Measurements on three cells, with applied current densities of 2.7, 3.5, and 9.3 peq·cm^{-2}·s^{-1}, respectively. The effluxes during current application were estimated via the Goldman (1943) flux equation as 0.09, 0.00, and 0.46 pmol/cm^{-2}·s^{-1}.

∥∥ Same as but on a different batch of cells.

The mean conductance of 8 μmho·cm^{-2} of DNP cells can be approximately accounted for in the following way: The K$^+$ conductance is reduced by 82% (T_K measurements), from 23 to 4 μmho·cm^{-2}, while the remaining 5 μmho·cm^{-2} is attributed to the conductances of the other ions, apparently unaffected by the DNP. The latter is probably not strictly true because the resting Cl$^-$ conductance is considerably increased by the DNP treatment (Table II). The absolute value

of the resting Cl^- conductance is still quite small, however, and presumably the Cl^- conductance under applied voltage is similarly small. The overall decrease in membrane conductance due to the DNP treatment can thus be essentially accounted for by the change in the K^+ conductance alone.

The enhancing effect of DNP on both the influx and efflux of Cl^- (Table II) was unexpected because it is generally assumed that these fluxes are completely independent of one another and have different characteristics. The energy requirement for net Cl^- influx is about 5 $kcal \cdot mol^{-1}$ thus pointing to an active transport mechanism, whereas Cl^- efflux seems to be passive (cf. Spear et al., 1969). The Cl^- efflux values obtained on control cells during hyperpolarization are also consistent with a passive Goldman-type behavior. A net Cl^- efflux of 0.9 $pmol \cdot cm^{-2} \cdot s^{-1}$ occurred during the DNP treatment.

In summary, DNP had six measurable effects on the *Nitella* membrane: (a) inhibition of inward K^+ movement; (b) depolarization of the membrane; (c) increase of membrane resistance; (d) decrease of T_K; (e) enhancement of Cl^- transfer in both directions; and (f) rendering of the membrane passive especially with respect to K^+ movement and its effect on E_m.

The time-course of the effect of DNP on the membrane resistance and

TABLE III
EFFECT OF EXTERNAL K ON DNP-POISONED CELLS

Solution	E_m	E_K
	mv	*mv*
K solution (1 mM K^+)	156 ± 14.4	111
K solution +200 μM DNP (1 mM K^+)	87 ± 10.9	111
K solution +200 μM DNP +4.5 mM K_2SO_4 (10 mM K^+)	55 ± 6.8	52

potential suggests an indirect effect rather than a rapid direct effect on the membrane. The question of DNP as a proton carrier is discussed later.

Effects of pH 4.7 on Membrane Properties

The lowering of the pH of the K solution from 5.7 to 4.7 resulted in Cl^- fluxes very similar to those obtained with 50 μM DNP at pH 5.7; influx was 0.9 $pmol \cdot cm^{-2} \cdot s^{-1}$ whereas efflux ranged between 1.4 and 1.9 (Table II). The effect of low pH on the K^+ fluxes was slightly less severe than that of DNP: influx and efflux became approximately balanced at about 0.45 $pmol \cdot cm^{-2} \cdot s^{-1}$. Moreover, depolarization at pH 4.7 to about -100 mV occurred, about the same as with DNP. However, the membrane conductance was not significantly changed by the low pH treatment; it was typically about 60 μmho $\cdot cm^{-2}$, ranging between 30 and 100 (Table II).

The apparent indifference of the conductance to pH can possibly be accounted for by an increase in H^+ conductance about equal to the decrease in K^+ conductance. The latter is concluded from the decreased resting K^+ influx and the correlation found between the resting and applied K^+ currents as reported above. An appreciable H^+ conductance at pH 4.7 appears possible in view of the large resting H^+ influx of 8 $pmol \cdot cm^{-2} \cdot s^{-1}$ observed at this pH (Rent et al., 1972; Barr et al., 1974). Chloride is another possible contributor to the measured

conductance at pH 4.7, but the calculated value obtained from its resting fluxes is so low as to suggest that its contribution would be small.

DISCUSSION

Current thought concerning the origin of the resting potential in many plant cells focuses on the idea of electrogenic H^+ pumping (Slayman, 1965; Kitasato, 1968; Spanswick, 1972, 1973; Vredenberg and Tonk, 1973; Brown et al., 1973; Richards and Hope, 1974). Recently the discrepancy observed between tracer
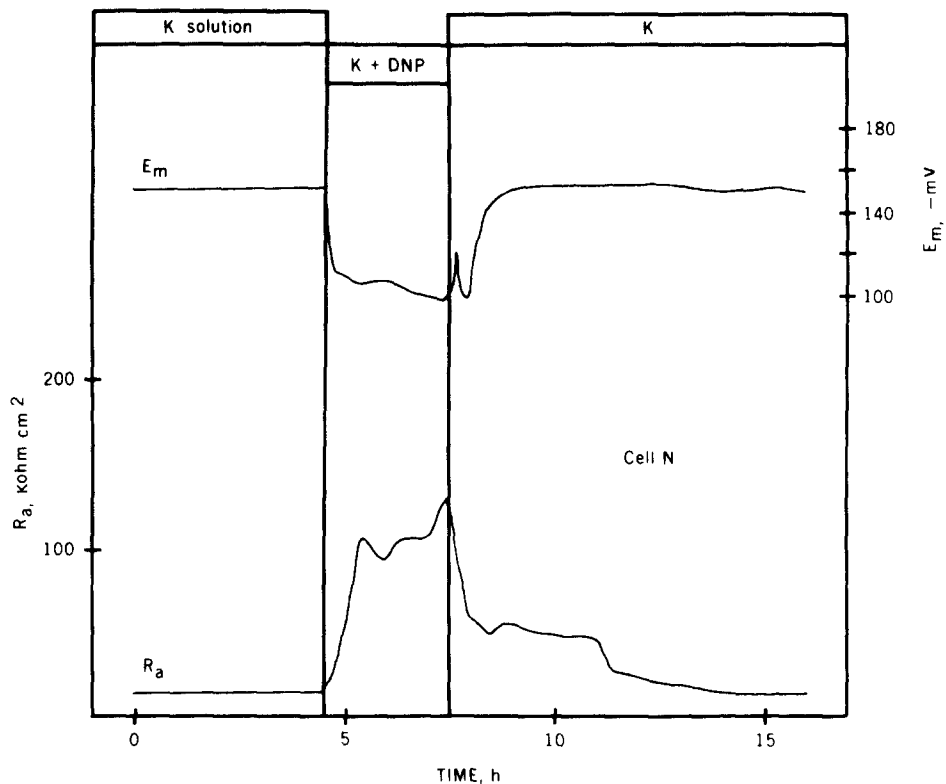

FIGURE 5. Effect of 50 μM DNP on the rest potential and membrane resistance of a 6-day-old cell of N. clavata. A transient hyperpolarization after the removal of DNP was observed in two of the four cells studied.

fluxes of K^+, Na^+, and Cl^- and the electrically measured conductance of the membrane has come to be interpreted as an externally induced H^+ flux through this electrogenic component (Spanswick, 1974). It is generally recognized that electrogenic ion pumps do not work independently of an externally applied current. The basis for this is that the ion pump has its own intrinsic resistance (Finkelstein, 1964; Rapoport, 1970). Slayman (1965) also described a circuit model for the electrical properties of the Neurospora crassa membrane where the electrogenic pump is in series with a resistance separate from the normal passive resistance of the membrane (also see reviews: Lüttge and Putman, 1976).

In a typical result from the present work a hyperpolarizing current of 0.7 $\mu A \cdot cm^{-2}$ caused the membrane potential to rise from -150 to -170 mV, and the inward K^+ current to increase from 1.6 to 6.0 $pmol \cdot cm^{-2} \cdot s^{-1}$. This increment in the K^+ flux can account for 57% of the applied current assuming the large net K^+ influx and balancing H^+ efflux remain unaffected. This may be unlikely if indeed the current is traveling through the mechanism responsible for the resting net K^+ influx as suggested below. If hyperpolarizing the membrane interferes with H^+ extrusion, which is normally balanced by the K^+ influx, then 90% of the applied current can be accounted for under our conditions. The large jump in K^+ influx represents a discontinuity in the flux behavior, i.e., the increase is much greater than that expected from the increase in driving force for K^+ movement through a passive channel. The latter, as defined by $E_m - E_K$ rises from 40 to 60 mV, or 50%. A similar picture emerges when the Goldman flux equation is used. Thus, it appears that only a sharp increase in conductance or permeability can explain the large K^+ current if conventional interpretations are adhered to.

In DNP-poisoned cells the electrogenic component of the membrane potential is eliminated, net K^+ uptake is eliminated, the electrical resistance of the membrane is increased to within the range predicted from passive fluxes, and the large K^+ current during hyperpolarization of the membrane is absent. This strongly indicates that the mechanism responsible for both types of K^+ movement studied, i.e., the resting K^+ influx and the inward K^+ current under applied voltage, may be one and the same. The correlation we observed between this magnitude of the resting K^+ flux and the transference number is also consistent with this interpretation.

A problem related to the use of DNP in this work is that DNP itself is a well-known hydrogen ion ionophore. This is also true for other metabolic uncouplers (e.g., carbonyl cyanide n-chlorophenylhydrazane and carbonyl cyanide-p-trifluoromethoxyphenyl-hydrazane). Evidence arguing against DNP playing a direct role in the membrane properties studied here follows: the effect of 1 mM DNP on the electrical conductance of artificial phospholipid bilayers is many orders of magnitude less than the electrical conductance measured here (Bielawski et al., 1966). The passage of DNP through the *Nitella* cell membrane appears to be in the undissociated (uncharged) form as no detrimental effects of DNP were observed with DNP in the anionic form (high pH) (Barr and Broyer, 1966), and Slayman and Slayman (1970) have shown that DNP uncoupling of respiration in *Neurospora crassa* takes place before effects on the membrane potential and transport. It should also be pointed out that the effects of DNP are completely reversible at the concentration level used and that the average cell life expectancy in DNP is >16 days at a light intensity of 2,700 $ergs \cdot cm^{-2} \cdot s^{-1}$ (Barr and Broyer, 1966). This is extremely strong evidence for an indirect effect.

The data presented here showing the large inhibitable K^+ current, coupled with previous work establishing electrogenic H^+ pumping in the *Characeae* and the thought that much of an applied current may travel through electrogenic pumps, suggest that much of the observed K^+ current may be through an electrogenic H^+/K^+ exchange mechanism. Although direct evidence for tightly

coupled H^+/K^+ exchange is lacking, net uptake of K^+ with concurrent H^+ efflux has been observed in microorganisms (Rothstein, 1972), fibrous roots (Pitman, 1971), red beet (Poole, 1974), and leaf tissue (Nobel, 1969). This suggests a certain parallelism with Na/K exchange observed in animal cells. Recent work from this laboratory also suggests a close relationship between H^+ efflux and K^+ influx in *Nitella clavata* (Barr et al., 1977).

Here we have reported a mean K^+ influx of 1.73 $pmol \cdot cm^{-2} \cdot s^{-1}$ and an efflux of 0.14, with E_m averaging -152 mV. As stated in the Introduction the net K^+ influx has been interpreted as an inward current that balances H^+ extrusion, the latter having been stimulated by the passive loss of cellular NH_3 and subsequent acidification of the cell interior. This conclusion has been substantiated by more direct evidence of H^+ extrusion (Barr et al., 1977), i.e., actual acidification of the medium by *Nitella* cells that had been preloaded with H^+. The H^+ extrusion rate was 2.7 $pmol \cdot cm^{-2} \cdot s^{-1}$ when 1 mM K^+ was present in the medium and 11.9 for 20 mM K^+. This evidence supports the idea that a lowered internal pH favors H^+ extrusion, with the latter rate limited by the availability of external K^+ for exchange. Depolarization of the membrane by K^+ may also be a factor favoring H^+ extrusion. The stoichiometry of the H^+/K^+ exchange has not as yet been studied, but it does appear safe to assume that some coordination of the two ionic movements is present. The question of how the stoichiometry of the H^+/K^+ transfer may vary with the membrane potential remains unanswered; however, a ratio greater than one is necessary to account for the observed electrogenic component of the membrane potential, i.e., the difference between E_m and E_K.

There is no need to propose both active and passive K^+ channels to account for the behavior observed in the present study. Our findings suggest that a single mechanism may be responsible for both types of K^+ movement studied, i.e., the resting K^+ influx and the inward K^+ current under applied voltage.

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