H^+ AND K^+ TRANSPORT IN *NITELLA*

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INTRODUCTION

Ionic movehemts across the membrane of characean cells have conveniently been separated into two different components: active transport, requiring input of energy: and passive diffusion. Hope and Walker (1960, 1961), and Hope (1963), considered the active fluxes to be primarily responsible for maintenance of the concentration gradients while the passive fluxes represented the drift toward equilibrium and determined the level of the membrane potential.

This is true as long as the active components result in no net charge transfer, or, in other words, are electroneutral.

If true, the diffusion potential developed across the selectively permeable membrane can be calculated using the Goldman equation:

$$E_m = \frac{RT}{F} \ln \frac{(P_K K_o + P_{Na} Na_o + P_{Cl} Cl_i)}{(P_K K_i + P_{Na} Na_i + P_{Cl} Cl_o)}$$

R = universal gas constant, 1.99 cal/mole/degree
T = absolute temperature, ^oK
F = Faraday constant, 23,060 cal/mole/volt
P = permeability coefficient, cm/sec.
K_i, K_o, etc. = molar concentration of the ion inside and outside the cell, respectively
E_m = resting potential of membrane, volts

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The Goldman equation shows that the concentrations of diffusable ions in contact with the cell membrane control the level of the membrane potential. The principle ions of characean cells are, in fact, K^+, Na^+, and Cl^- which account for ninety percent of the total intracellular electrolytes.

A summary of studies using variable external ionic concentrations has been compiled by MacRobbie (1970). In each case, the measured membrane potential was within the limits predicted by the model for passive diffusion when the external K^+ concentration was 0.1 mM. Hope and Walker (1961) reported that variations in external K^+ and Na^+ concentrations affected the potential in a way consistent with the Goldman equation but only in the absence of external Ca^{++}. Spanswick, Stolarek, and Williams (1957) found that when external K^+ concentration was increased in the presence of Ca^{++}, the potential did not follow a Goldman relationship. Kitasato (1968) and Spanswick (1972) have measured the effect of 1.0 to 100 mM external Na^+ on the resting potential and found little or no effect. Nitella clavata in a solution containing 1.0 mM K^+ has a potential about 40 mV more electronegative than E_k, the potassium equilibrium potential, indicating a non-Goldman behavior (Rent, Johnson and Barr, 1972). Spanswick (1972) has reported similar findings on Chara corallina.

Some of these difficulties could be resolved by resorting to a "pump-leak" hypothesis in which ions are pumped as net charge, balanced by a passive return current. However, there still existed a discrepancy between the electrically measured conductance and the conductance

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calculated from flux measurements. As stated by Williams, Johnston, and Dainty (1964) electrical conductance should be related to passive fluxes since ions carry the current flow when current is passed through the membrane by means of an external circuit. They found the measured conductance of <u>Nitella translucens</u> to be ten times the value calculated from K^+ and Na$^+$ fluxes. Such results have been consistently found in all subsequent studies on characean cells.

Kitasato (1968) proposed a hypothesis which seemed to explain the discrepancy. He suggested that the difference between measured conductance of characean cell membranes and that calculated from ionic flux measurements could be accounted for if the membrane is significantly permeable to H$^+$. When the membrane potential was clamped at E_k, and the external solution pH was changed from 6 to 5, Kitasato (1968) observed that the current required to maintain the potential at E_k was consistent with a net positive influx. In light of the facts that partial conductance calculated from K^+ and Cl$^-$ fluxes were very small compared to the measured conductance and only the external pH was varied, he concluded that H$^+$ must account for the major portion of membrane conductance. An increased Na$^+$ permeability was ruled out on the basis of the small effects which an increased external sodium concentration has on the membrane potential.

Kitasato (1968) thus proposed an electrogenic H$^+$ efflux pump in combination with the passive H$^+$ influx; by this pump-leak mechanism the level of the membrane potential would be determined. However, the primary function of the H$^+$ pump would be to maintain the intracellular pH at the

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proper level in the face of the considerable inward passive flux of H^+.

Spear, Barr and Barr (1969) have obtained indirect evidence for electrogenic H^+ extrusion by Nitella. They reported the presence of macroscopic acid bands on the external surface of the internodal cell, these alternating with alkaline bands; also, membrane potentials more negative than could be accounted for by the diffusion of any major ion were reported. Estimates of H^+ extrusion from their work are very close to that suggested by Kitasato (1968); these were $5-20 \times 10^{-12}$ mole cm^{-2} sec^{-1} as compared to the 40×10^{-12} mole cm^{-2} sec^{-1} proposed in Kitasato's work based on membrane conductance.

Lucas and Smith (1973) have measured the pH along the length of the acid and alkaline regions of Chara corallina and found 10^4 fold changes in H^+ concentration between the two regions. The large differences found in the alkaline regions led them to conclude that a localized OH^- efflux is superimposed on an apparently uniform H^+ efflux system. Lucas (1976) has subsequently demonstrated that the OH^- efflux has a 1:1 stoichiometry with active bicarbonate uptake and that the bicarbonate uptake is probably electrogenic. This has to some extent shifted the emphasis away from the idea of an electrogenic H^+ extrusion pump, but it is very possible that both active transport mechanisms are present, operating under different conditions: the OH^- efflux, which is downhill, would operate mainly above pH 7, (bicarbonate present), while the H^+ extrusion pump would operate below pH 7.

Evidence for the latter was obtained by Barr, Holland, and Bower (1977) on Nitella cells which were pre-loaded with H^+ by soaking them in a

solution of low pH, 4.70. Such cells were observed to extrude net H^+ at a rate exceeding 10^{-11} mole cm^{-2} sec^{-1} when exposed to a solution containing 20mM K^+.

Raven and Smith (1974) have argued that the primary role of H^+ extrusion is internal pH control. Others have suggested and provided evidence that H^+ extrusion may also play a predominant role in electro-genesis (Slayman, 1970), (Spanswick, 1973), (Higinbotham, 1970) and Rent, Johnson and Barr (1972). A third possible role for H^+ extrusion, as suggested above, is its participation in active K^+ uptake through some type of K^+/H^+ exchange mechanism. Poole (1974) has reported evidence for the K^+/H^+ exchange mechanism operating in red beet tissue. Slayman and Slayman (1968) have described a comparable system in Neurospora in which K^+ uptake appeared to be coupled to the extrusion of both Na^+ and H^+. Of course, for such mechanisms to be electrogenic the exchange ratio must be different from unity. It is possible that H^+ extrusion serves in all three functions listed above: internal pH control, electrogenesis, and K^+ uptake. If internal pH control is the primary function of H^+ extrusion, a variable K^+/H^+ exchange ratio could serve to modulate the level of the resting potential, (Poole, 1974; Ryan, Barr, and Zorn, 1978).

Proposed Studies

The present work represents an extension of the studies of Barr, Holland, and Bower (1977) and of Ryan et al. (1978).

The main objective is to attempt to determine if a fixed stoichio-metry of the K^+/H^+ exchange is present.

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MATERIALS

Culture and harvest of Nitella cells

Nitella clavata was cultured in either glass or plexiglass 3-liter
containers or 12-liter glass tanks with constant aeration under illumina-
tion of approximately 2000 lux measured at the culture solution surface,
from Sylvania Gro-Lux[R] and Cool-White[R] fluorescent lamps. Sixteen
hours of light were alternated with eight hours of darkness. The
composition of the culture solution is given in Table 4.

The harvesting of internodal cells, the second or third from the
growing tip was done by excision of adjacent internodal and branch cells.
The harvested cells ranged from 3-5 centimeters in length and were 700-900
micrometers in diameter.

Standard pre-conditioning solution

The isolated cells were placed in K solution, the composition of which
in millimoles per liter was 1.0 KCl, 0.1 NaCl, 0.1 $MgCl_2$, and 0.1 $CaCl_2$.
The pH of this solution was 5.70. The cells were kept in a Precision
Scientific (model 805) incubator at $22^{\circ}C$ and under 350 lux Cool-White[R]
fluorescent illumination. The cells were pre-conditioned for seven days
under the above conditions prior to experimentation.

Vacuolar ionic concentrations

The seven day old cells had an average vacuolar K^+ concentration

of 75 mM and an average vacuolar NH_4^+ concentration of 25 mM. The former was determined by analysis of 5 microliter samples of the vacuolar contents using a Perkin-Elmer Model 303 Atomic Absorption spectrophometer with flame detector. The NH_4^+ concentration was determined by using a modified Nessler's method.

COMPOSITION OF CULTURE SOLUTION

Concentration	Material
0.4 mM	KNO_3
1.0 mM	$MgSO_4$
1.0 mM	$NaCl$
1.0 mM	$NaHCO_3$
1.5 mM	$CaCl_2$
0.01 mM	Na_2MoO_4
0.0037 mM	H_3BO_3
0.01 mM	NaH_2PO_4
0.2 mM	KCl
5 ppm	Streptomycin
0.67 ml/liter	Micronutrient solution IV-35

COMPOSITION OF MICRONUTRIENT SOLUTION

14 g humus soil boiled for one hour in 200 ml of 10 mM nitrilotriacetic acid neutralized to pH 9.2

Culture solution base was distilled and deionized water.

H^+ efflux measurements

H^+ extrusion was measured with a Leeds and Northrup pH meter using a small volume pH electrode. Experiments continued for 0.75-8 hours, depending on the requirements of the experiment. Prior to the measurement of H^+ extrusion, the cells were pre-loaded with H^+ by placing them in a large volume of K solution at pH 4.70, usually for a period of 85 minutes. This allowed them to take up a total of 1-5 x 10^{-8} mole H^+ per cm^2 of cell surface. Cells were then rinsed in fresh K solution at pH 5.70 for a period of 3 minutes, followed by a second rinse in fresh K solution for 17 minutes. After this 20 minute rinse period the net H^+ efflux in K solution was found to be negligible. The cells were then placed in 20 ml of a solution designated as 20X K solution; this contained KCl, NaCl, $CaCl_2$ and $MgCl_2$. each at 20 times the concentration present in K solution. Net H^+ extrusion was then measured by addition of 5 mM KOH at frequent intervals to keep the pH at 5.70. Quick dispersion of the KOH was accomplished by means of a 10 ml pipette positioned with the tip immersed in the K solution. Attached to the pipette was a 10 cc syringe which was used to continuously draw the K solution up into the pipette and then return it to the test vessel. Care was taken not to damage any cells. This procedure was continued until the rate of the pH change of the external K solution became negligible.

$\underline{K^+ \; fluxes}$

K^+ fluxes were measured with ^{42}K at an initial specific activity of 2 Ci mole^{-1}. Approximately 2.4 x 10^{-4} moles of tracer was available for each experiment.

K^+ influxes were measured using the same system as that defined under H^+ efflux measurements with the exception that the 20X K solution was 20X ^{42}K solution.

Cells were placed in the 20X ^{42}K solution and removed in groups of five at 10, 20, 30, 70, and 120 minutes. Each cell in each group was given two quick dips in cold 20X K solution and then placed in fresh 20X K solution for a thirty minute rinse before being radioassayed. Radioactivity was then assayed by placing the cell in a specially designed holder directly beneath a Geiger-Muller tube. Counting was done with a Nuclear-Chicago 8703 decade scaler. Appropriate corrections for geometry and coincidence were made. (See appendix).

K^+ effluxes were measured, but the data were not interpretable because it was not possible to determine the specific activity of the effluxing K^+ with any accuracy. This was due to a high ^{42}K concentration in the cell wall and/or cytoplasm which did not rinse out quickly. Thus, specific activity of the cell interior could not be calculated since most of the ^{42}K measured was exterior to the protoplast rather than within it. Under the protocol used it was not possible to obtain the desired information.

$\underline{Cl^- \; influx}$

Cl^- incluxes were measured using the same procedure as that described

under K^+ fluxes with the exception that the 20X K solution contained $^{36}Cl^-$. The correction factors for geometry and self-absorption were taken from Bower (1977).

Initial specific activity of $^{36}Cl^-$ was 0.24 Ci mole^{-1}, Approximately 2.1×10^{-3} moles of tracer was available for each experiment.

Membrane Potential Measurements

Membrane potentials were measured with the cell resting in a narrow plexiglass trough (60 x 8 x 3mm). The experimental solution, flowing at a rate of 2 ml/min. was delivered by gravity into the trough from a reservoir via polyethylene tubing. The solution was removed with a filter paper siphon. The entire volume of the trough was replaced approximately every two minutes.

The Ag^+/AgCl glass microelectrode consisted of a chlorided silver wire inserted into a 1.0 mm microcapillary glass tube pulled to a tip diameter of approximately 10 um by a vertical pipette puller, (David Kopf Instruments, model 700C). The chlorided silver wire was made by first removing the surface corrosion by sanding and then chloriding approximately 2 cm of the tip. The latter was accomplished by immersing about 2 cm of the tip in 1.0 M KCl solution, connecting the free end of the silver wire to the anode of a 1.5 volt battery and passing current for about 30 seconds. The return current was carried by a nichrome wire immersed in the same solution and placed about 1 cm from the immersed tip of the silver wire. The micro-capillary glass tube was filled with "artificial cell sap" consisting of 80 mM KCl, 20 mM NaCl and 5 mM $CaCl_2$. This cell sap simulates the vacuolar

contents of the cell and reduces the possibility of a diffusion potential

arising at the tip.

A similar but larger electrode was used for reference. This reference

electrode was capped with a rubber bulb. A chlorided silver wire, (as

described above), was inserted through the capped end. The microelectrode

and the reference electrode were connected to a Keithly 604 differential

electrometer. The electrometer output was delivered to the input of a

Leeds and Northrup Speedomax XL 680 strip chart recorder. It was found

that electrical artifacts were minimized by using the differential inputs

of the electrometer and grounding the trough to earth through a Ag^+/AgCl

electrode similar to the reference electrode. "Tip" potentials between the

reference and measuring electrodes were measured by placing the reference

electrode in the experimental solution and the measuring electrode into

artificial cell sap solution, with a filter paper salt bridge between the

two solutions; these were subtracted from the measure values in each case

to give the potential for the cell itself. Values obtained in this manner

were usually -10 to +10 mV.

Following insertion of the measuring electrode the cell was given time

to recover and stabilize. The solution flowing through the trough during

this period of stabilization was K solution at pH 5.70.

A stabilized cell was then pre-loaded with H^+ by exposing it to K

solution at pH 4.70 for a period usually lasting 85 minutes. This was

followed by a twenty minute rinse period with K solution at pH 5.70.

Following the rinse the solution bathing the cell was changed to 20X K

solution. At the termination of the 20X K period, the solution was returned to K solution at pH 5.70. Variation in the membrane potential was recorded during this entire sequence.

It should be noted that the membrane potential, (E_m), is actually the potential across the cell wall, cell membrane, and vacuolar membrane in series, (the microelectrodes are inserted into the large center vacuole), but for convenience is referred to as the membrane potential. The potential across the vacuolar membrane is usually about +15 mV, (the cytoplasm is more electronegative than the vacuole, presumably because of a Donnan effect).

Vacuolar pH Determinations

The pH of the vacuolar sap of Nitella cells was determined by first pricking the cell with a needle and subsequently drawing the fluid up into a 5 μ liter pipette. This fluid was then deposited on a slab of paraffin wax. When the latter was done the fluid remained in a droplet approximately 3 mm in height. A glass pH small volume electrode was then inserted into the droplet from above and the pH was read.

Relationship of K^+ influx to H^+ extrusion

The time course of H^+ release as seen in two separate experiments with <u>Nitella</u> cells when they are placed in 20X K solution is shown in Figure 1. In addition the corresponding K^+ influx for Exp 43-B is shown (see Figure 1). It is clear that much variability exists in both the total amount of H^+ released and the rate of H^+ release. A nearly eight-fold difference in the amount of H^+ released is seen under presumably identical conditions. In addition, the rate of H^+ release in experiment 41-B is approximately seven times that in experiment 43-B during the same initial 30-minute interval. During the second half of the first hour of extrusion the difference in H^+ efflux rates is much less. This variability in H^+ efflux rate in response to the same external K^+ concentration may possibly be related to the amount of H^+ taken up prior to the measurement of the K^+ induced H^+ efflux. This aspect is further discussed below.

It is to be noted that the "K^+ uptake" (see Figure 1) is actually the tracer-measured K^+ influx and is, therefore, the relevant flux if the behavior is to be explained solely in terms of an obligate K^+/H^+ exchange (antiport) system. It is clear that an overall 1:1 exchange does not exist and suggests that some third ion must participate in order to achieve charge balancing. This becomes apparent when noting that the amount of K^+ taken up and its corresponding rate is much greater than that of H^+. The H^+/K^+ exchange ratio is therefore less than 1/2 (see Figure 1). Still

left open is the possibility that some of the K^+ enters the cell passively, i.e., not by the antiport mechanism. The charge-balancing may be effected through net Cl^- influx as treated below.

Two experiments were conducted under somewhat different conditions in which an attempt was made to account for the main balancing of the ionic currents through the membrane during the H^+/K^+ exchange. It is clear that the unidirectional K^+ influx exceeds H^+ extrusion (see Figures 1 and 2). If a net Cl^- uptake occurs concurrently, it will tend to support the idea that some of the K^+ entry is balanced by Cl^-. It is to be noted that K^+ is by far the predominant cation in the solution, being 10-fold higher in concentration than Na^+.

The external solution in some experiments was 28X K rather than 20X K (see Figure 2). Chloride was at 42 mM in the 28X K solution. A net Cl^- uptake was demonstrated (Figure 2) with the corresponding net influx being 5.33×10^{-12} mole cm^{-2} sec^{-1}. This value was obtained by subtracting the (relatively small) unidirectional efflux obtained in another experiment from the unidirectional influx measured here. The value of the efflux is, perhaps, an approximate one since it comes from another experiment, but because of its small value, it does not substantially affect the conclusion that a net Cl^- influx of a few pmol cm^{-2} sec^{-1} occurs. It Therefore appears certain that a net influx of KCl does occur under these conditions in addition to the H^+/K^+ exchange. These results are similar to those obtained by Nakagawa et al (1974), on Nitella flexilis.

Variability of H^+ extrusion

The variability of H^+ extrusion in a number of experiments, where the major difference in conditions was the duration of the low pH pre-treatment, is shown (see Table 1). Our data do not indicate any correlation between the magnitude of H^+ extrusion and the duration of this low pH. The most frequently used time period for the low pH pre-treatment was 85 minutes (see Table 1). In all earlier experiments cells were titrated to pH 4.70 with 5 mM HCl in order to determine the temporal characteristics of the net H^+ uptake. The titration was terminated when the H^+ uptake became negligible; the total time varied considerably from one experiment to another. The total amount of H^+ taken up in this earlier work varied between 3.6 x 10^{-8} mole/cm^2 and 10.9 x 10^{-8} mole/cm^2 while H^+ uptake in later studies (see Table 1) varied between 6.4 x 10^{-8} mole/cm^2 and 10.9 x 10^{-8} mole/cm^2. Due to the general consistency of the uptake during the first 85 minutes of low pH pre-treatment, this period was selected as the optimum time to achieve maximum H^+ uptake while minimizing the possibility of damage to the cells. In almost every case the H^+ uptake after 85 minutes at pH 4.70 became very slow.

The only variable that does appear to affect the total amount of H^+ extruded and the magnitude of the extrusion rate is the total amount of H^+ taken up by the cells during the low pH treatment.

In five of the seven experiments shown (see Table 1) the amounts of H^+ extruded in the first hour are about the same, 4-6 x 10^{-8} mole/cm^2; in these same five experiments the total H^+ uptake was 9-11 x 10^{-8} mole/cm^2.

Thus, these cells were very consistent in their behavior: they extruded about 1/2 of the accumulated H^+ during the first hour in the 20X K solution.

The only cells that had poor H^+ extrusion were those which had somewhat lower total H^+ uptake (experiments 35 and 43B). This seems to suggest that a critical amount of H^+ must accumulate within the cell before appreciable H^+ is available for extrusion. A probable contributing factor is the large size of these poorly extruding cells: the cells in experiment 35 and 43B had diameters 40% and 25%, respectively, greater than those in other experiments. This means that the volume available for storage of H^+ was greater. The amounts of H^+ taken up per unit volume in these larger cells were only 47% and 57%, respectively, of those in the more usual sized cells.

Our results also seem to indicate that the duration of the rinse period, (K solution), prior to the 20X K period has some effect on the first hour of extrusion (see Table 1). In experiment 41-A where a three minute rinse was used, the H^+ extrusion was somewhat smaller than that for cells rinsed twenty or forty minutes. This factor does not come into play in considering the main results since in the latter a standard 20-minute rinse was used.

Location of H^+ storage

In an attempt to determine where the H^+ taken up was stored the vacuolar pH was measured on cells before and after exposure to the low pH. These

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measurements indicated an average vacuolar pH of 5.50 for the control cells and 4.60 to 4.90 for cells exposed to low pH for 14 to 21 hours. These results indicate that at least some of the H^+ being taken up is indeed moving to the vacuole and not only to the cytoplasm or binding on the cell wall.

Maximum H^+ efflux

Excluding those cells that are either very large or that had only a 3-minute rinse, the initial 30-minute H^+ efflux was consistently large at 24 to 29 x 10^{-12} mole cm^{-2} sec^{-1} (see Table 1). It would appear that this is the maximum rate at which Nitella cells can extrude H^+.

Relation of membrane potential changes to the H^+ and K^+ fluxes

Because of the variability in H^+ extrusion under presumably very similar conditions as well as the variability in the K^+ influx, a variable behavior of the membrane potential under the same set of conditions is not unexpected.

Cells were subjected to the sequence of low pH, rinse, and high K^+ solutions, similar to the conditions used for flux measurements (see Figure 3). The difference in procedure was to use a flowing solution instead of maintaining the solution at a given pH via titration.

These results are inconclusive. In some cases the membrane potentials are greatly depolarized by the 20X K solution to below -40 mV whereas in experiment 18-2 a potential of about -120 mV is maintained for about 13 hours. This latter result is similar to that previously reported by Barr,

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Holland, and Bower (1977).

On this basis it seems highly unlikely that in the experiments involving 25 to 30 cells that all the cells would behave electrically in the same way.

The relationship between K^+ influx and the level of the resting potential is consequently obscure. However the fairly consistent results obtained on H^+ extrusion suggest that there is no simple connection of this phenomenon with the membrane potential. This is not entirely unexpected since no fixed stoichiometry of K^+ influx and H^+ efflux was observed.

DISCUSSION

This work includes only the second report of net H^+ extrusion in

charracean cells, the first being a similar K^+-induced H^+ extrusion in

Nitella cells exposed to low pH pre-treatment for 21 hours (Barr, Holland,

and Bower 1977). The maximum H^+ extrusion rate reported in that work was

11.9 x 10^{-12} mole/cm^2/sec.

In the present work it appears that Nitella cells do have the capa-

bility to extrude H^+ vigorously, up to 29 x 10^{-12} mole/cm^2/sec. In nature

this is probably masked by concurrent OH^- efflux of about the same magni-

tude. In Chara, it is known that OH^- efflux and HCO_3^- influx have a 1:1

stoichiometry at alkaline pH (Lucas 1975). But in the present studies it

appears that a strict relationship of H^+ extrusion to K^+ influx is not

present. Some of the reasons for this are discussed below.

The results of the present work indicate that the Nitella cell may

require a critical amount of H^+ accumulation within the cell before any

significant quantity of that H^+ will become available for net extrusion to

the external environment. This may reflect the cells ability to assimilate

a specific amount of H^+ which it is then apparently reluctant to give up.

Only when surplus H^+ exceeds this critical level does extrusion follow at

a rapid rate. This observation indicates an apparent buffering capacity

of an average cell somewhere in the neighborhood of 5 x 10^{-8} moles. It

is not known whether buffering power is present in the protoplasm, in

addition to the vacuole. MacRobbie (1975) has reviewed the role of the

vacuole as a depot for unwanted acidity in relation to pH control of the

protoplasm. An accurate measurement of the protoplasmic buffering capacity would prove to be difficult since contamination of vacuolar and protoplasmic contents by each other during separation is almost inevitable.

Actual measurements were made as part of this work of the effect of low pH pre-treatment (14-21 hours) on the vacuolar pH. The significant drop in vacuolar pH observed following low pH indicates that at least some portion of the H^+ taken up by the cell finds its way to the vacuole. This lends support to MacRobbie's theory and to the explanation being suggested here. Since cells in this work were in most cases subjected to only 85 minutes of the low pH pre-treatment, the crucial measurement would be the vacuolar pH of those cells exposed to this shorter period of low pH. This measurement has yet to be made. However, since after 85 minutes of exposure to low pH, the H^+ uptake becomes minimal, it is reasonably safe to assume that the major drop in vacuolar pH would occur as a result of the H^+ uptake during these first 85 minutes.

The apparent effect of cell diameter on H^+ uptake and subsequent extrusion, mentioned in the results, may possibly be explained by simply stating that the larger cells take up less H^+ per unit surface area and that the amount of H^+ taken up is stored in a considerably larger volume. All cells, whether large or small, take up about the same amount of H^+, but subsequent H^+ extrusion seems to require that the concentration of the accumulated acidity, whether buffered or not, exceed a certain value.

As indicated above a strict relationship of H^+ extrusion to K^+ influx does not appear to be the case in this work. The stoichiometric relationship here is unbalanced, and the observed fluxes may be explained in a

number of ways. While it remains plausible that K^+ may enter the cell partially via a H^+/K^+ exchange, the remaining influxing K^+ observed in this work must be balanced by Cl^- entry. While the present data do not allow a direct analysis of the Cl^- movement during the 20X K period, it is, nevertheless, at least true that net Cl is entering the cell in combination with K^+ to some degree.

Because of the apparent complexity of the K^+ inward movement, no simple relationship of the membrane potential to H^+ or K^+ movement is to be expected. The variable results seen with respect to membrane potential measurements in this work seem to support this notion. A general trend may be seen as the tendency of high external K^+ to depolarize the cell to extremely low levels, but with the cell maintaining the ability to re-polarize to at least its original resting level, if not higher, upon return of the external solution to normal or baseline concentrations of K^+. This generalized behavior and apparent resilience of the cell under unnatural conditions indicates, at best, that the Nitella cell can be forced to extreme limits and still return to a "normal" state with no apparent damage or ill effects. While this is not in itself a very profound conclusion, it does point out the utility of the Nitella cellular system in studying ionic behavior under a variety of conditions.

It has been previously noted that at least some of the H^+ is stored in the vacuole. This, however, gives no indication as to the relative amount of H^+ stored in the vacuole, as compared with the total taken up, nor a clue to the mechanism of transfer of H^+ across the cytoplasm. Insight

into the possible modes of transfer for the above await further work in this area; however, the H^+/K^+ antiport system of transport reported in E. coli by Brey, Rosen and Sorensen (1980) may be a system worth looking at.

Walker and Smith (1977) have discussed the relation of H^+ extrusion to the membrane potential. The results of the present work lend further support to their basic conclusions: that there does exist, in characean cells, electrogenic H^+ transport and that a large current (largely of H^+) flows across the plasmalemma if the pump is working and if the membrane potential is displaced to a more negative value.

Further, Poole (1978) has reviewed the relationship of H^+ efflux to K^+ uptake in different types of plant cells. He indicates that it seems very likely that K^+ uptake is mediated directly or indirectly by the electrogenic H^+ efflux pump. This conclusion is based not only on the many observations of K^+/H^+ exchanges found in various types of plant cells but also on the fact that many treatments affecting the activity of the electrogenic pump have a parallel effect on K^+ uptake.

The results of this work agree reasonably well with these conclusions in that H^+ extrusion is definitely stimulated by a high external K^+ concentration. However, the 1:1 stoichiometry observed in some cells and tissues does not appear to be present in Nitella clavata. This may be a rather broad statement about this particular species. Further work must be done to determine if the observed phenomena is indeed characteristic of Nitella clavata.

Table 1. H^+ extrusion of _Nitella_ cells exposed to 20X K solution.

Exp. No.	Duration of pH 4.7 Pretreatment (minutes)	No. of Cells	Total H^+ Uptake 10^{-8} mol/cm^2	Total H^+ Extruded 1st Hr 10^{-8} mol/cm^2	30 Minute Initial Flux 10^{-12} mol/cm^2/sec	Time for 95% Observed H^+ Extrusion (minutes)	Flux at end of Experiment 10^{-12} mol/cm^2/sec
26	95	23	10.4	3.84	26.0	408	1.9
29	165	26	10.9	4.19	24.0	438	1.7
35	85	20	6.4	0.05	0.12	-	-
*41A	85	23	10.0	4.77	19.0	39	0.3
41B	85	23	9.0	5.40	29.0	43	1.7
**41C	85	24	9.7	5.88	26.0	48	0.4
43B	85	15	7.1	0.71	4.4	43	2.6

* 3 minute K solution rinse
** 40 minute K solution rinse
  All other: 20 minute K solution rinse

Table 2. Summary of H$^+$ extrusion by _Nitella_ cells under the experimental conditions listed.

Exp. No.	Mean E_m, -mv Pretreatment K soln pH 4.70	Maximum E_m, -mv Rinse K soln pH 5.70	Minimum E_m, -mv 20X K soln pH 5.70	Mean E_m, -mv K soln pH 5.70
18-2	128	180	36	158
18-4	80	146	30	157
28	79	114	7	139
31	88	129	32	142
Mean	94	142	26	149

Table 3. ^{42}K Geometry correction factor and cell length for Nitella. Geometry correction factor = cpm of cell dispersed in 1.0 ml "killing solution"/cpm in live cell.

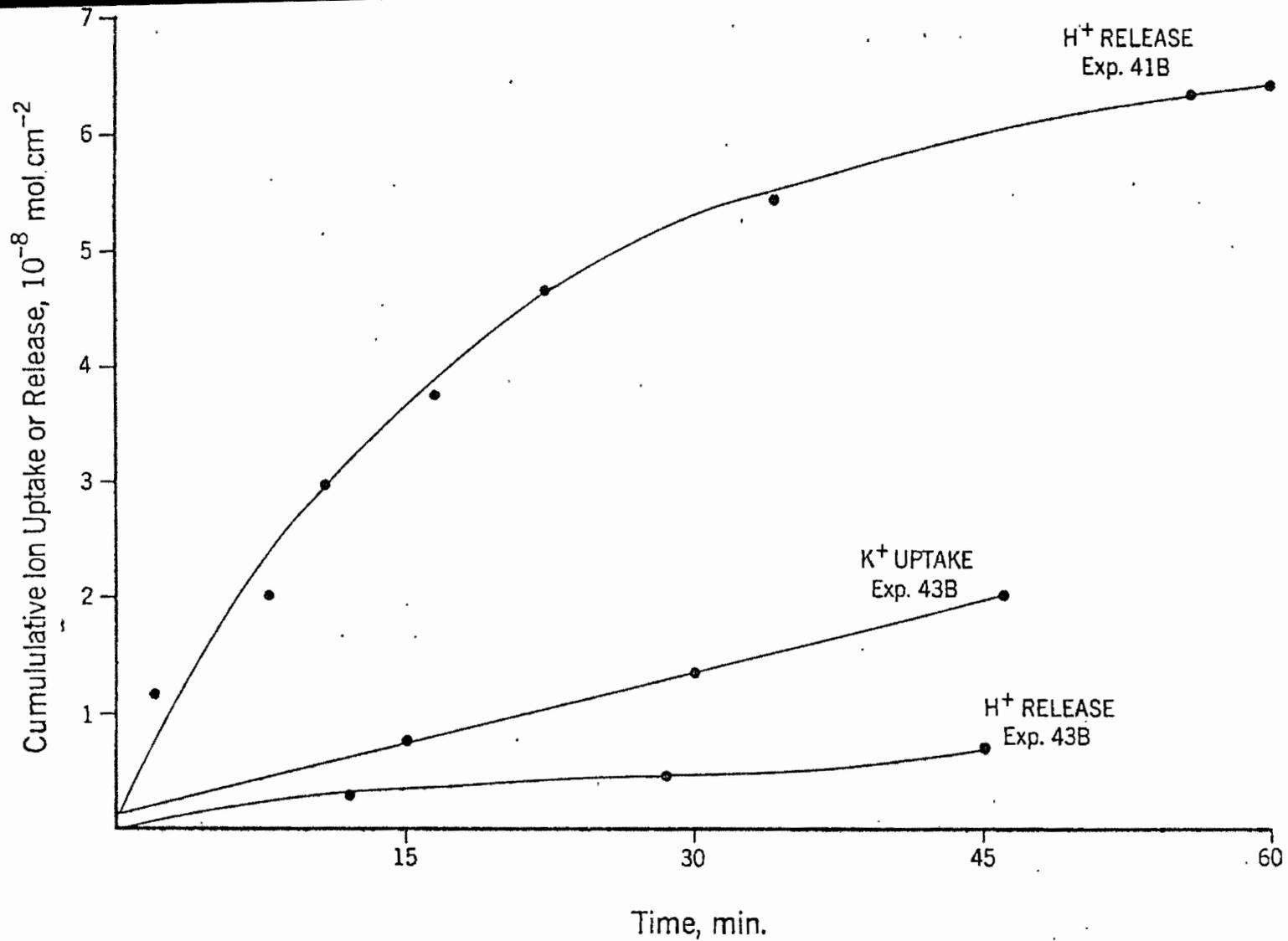| Cell Length, cm	^{42}K Correction Factor
2.0	
2.1	
2.2	
2.3	
2.4	
2.5	.552
2.6	.562
2.7	.573
2.8	.583
2.9	.594
3.0	.604
3.1	.615
3.2	.626
3.3	.637
3.4	.647
3.5	.658
3.6	.668
3.7	.679
3.8	.689
3.9	.700
4.0	.710
4.1	.721
4.2	.732
4.3	.742
4.4	.753
4.5	.764
4.6	.775
4.7	.785
4.8	.795
4.9	.806
5.0	.816
5.1	.827
5.2	.837

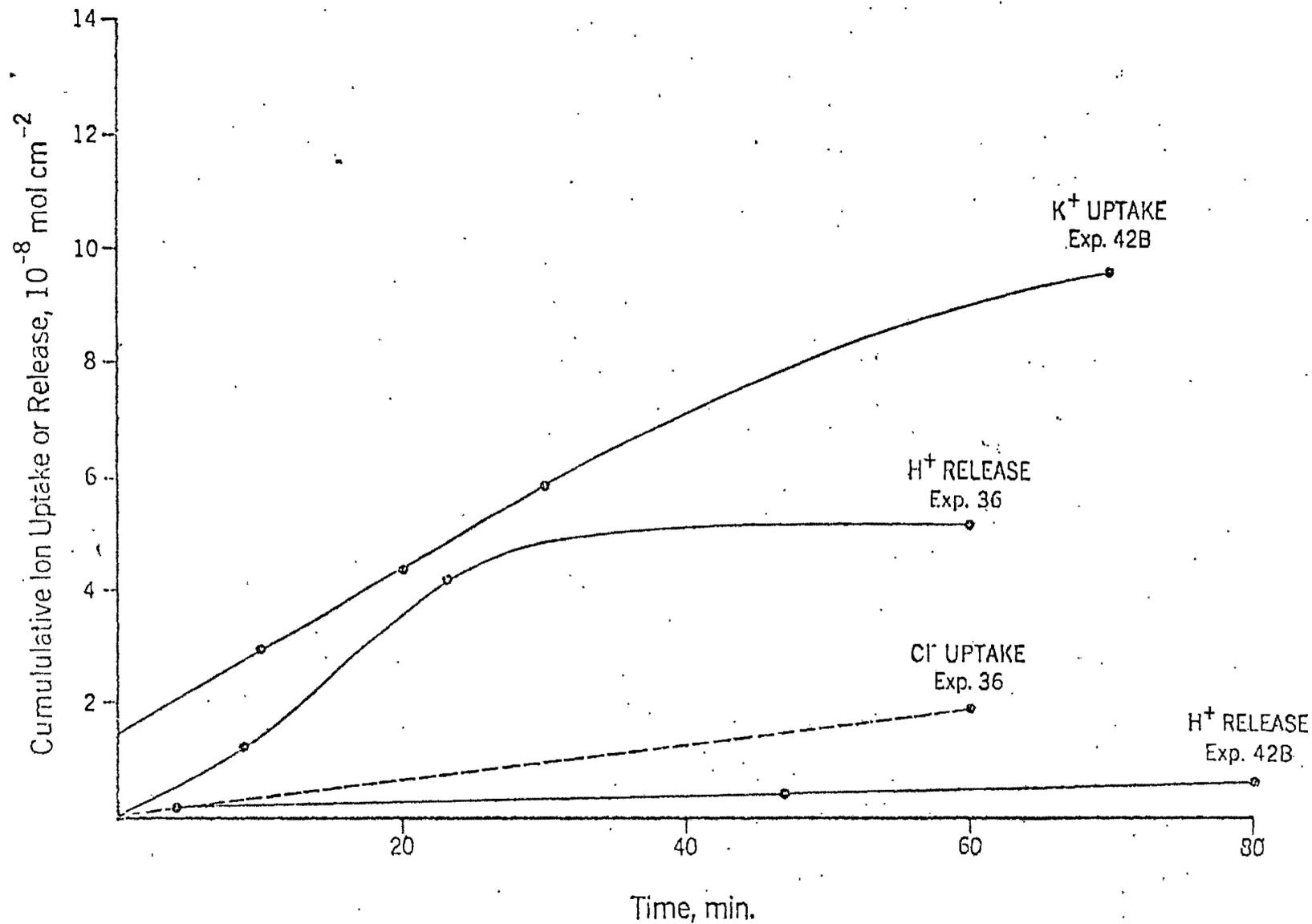Figure 1. Time course of K^+ uptake and H^+ release by <u>Nitella</u> cells during exposure to 20X K solution.

Figure 2. Time course of K^+ and Cl^- uptake and H^+ release by <u>Nitella</u> cells during exposure to 28X K external solution.
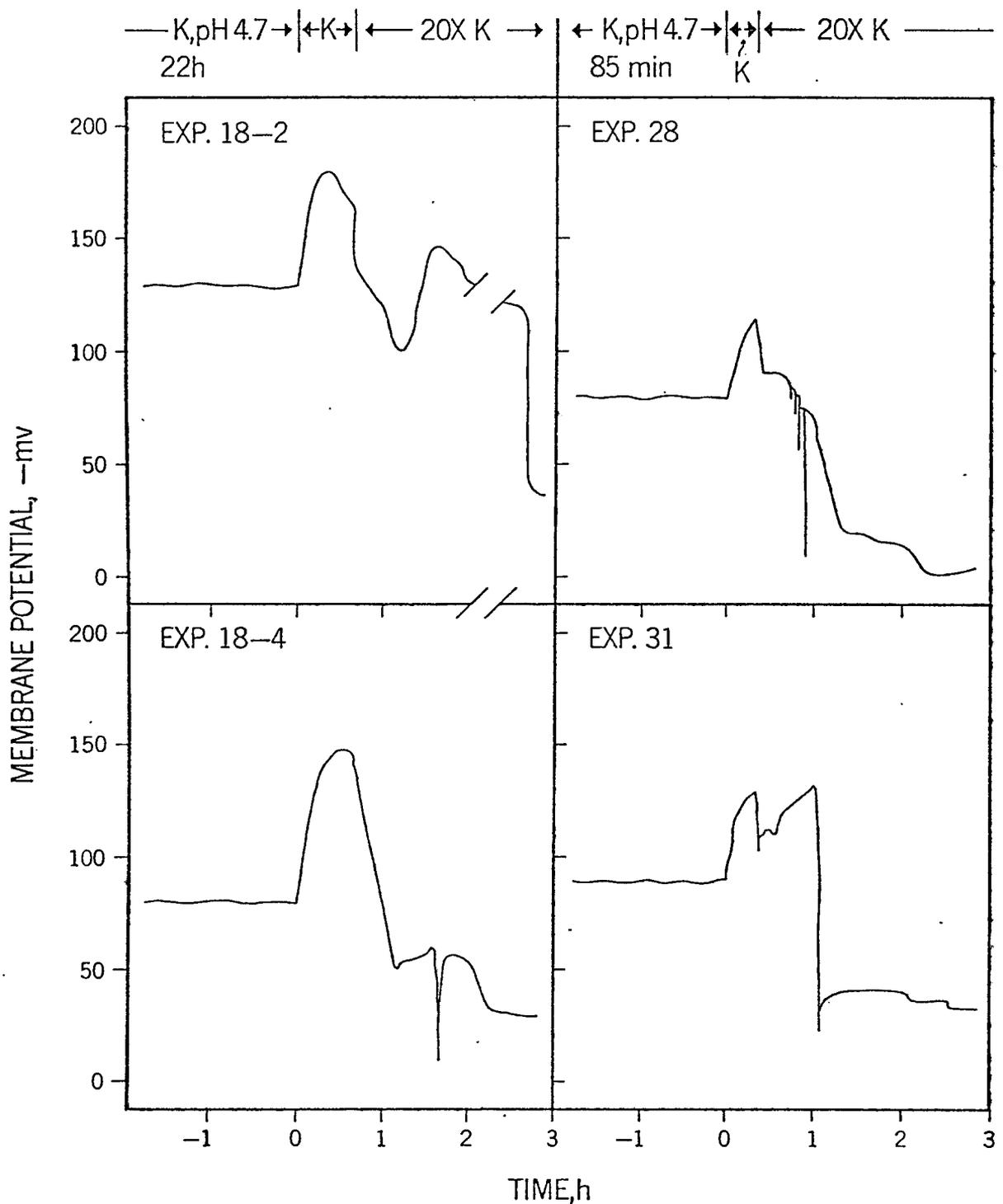
Figure 3. Time course of changes in the membrane potential of Nitella cells when they are exposed to the 20x K solution following a low pH treatment (K solution acidified to pH 4.7) and a rinse in K solution, pH 5.7. In Experiments 18-2 and 18-4 the duration of the acid treatment was 22 hours while in Experiments 28 and 31 the acid treatment was for 85 minutes.
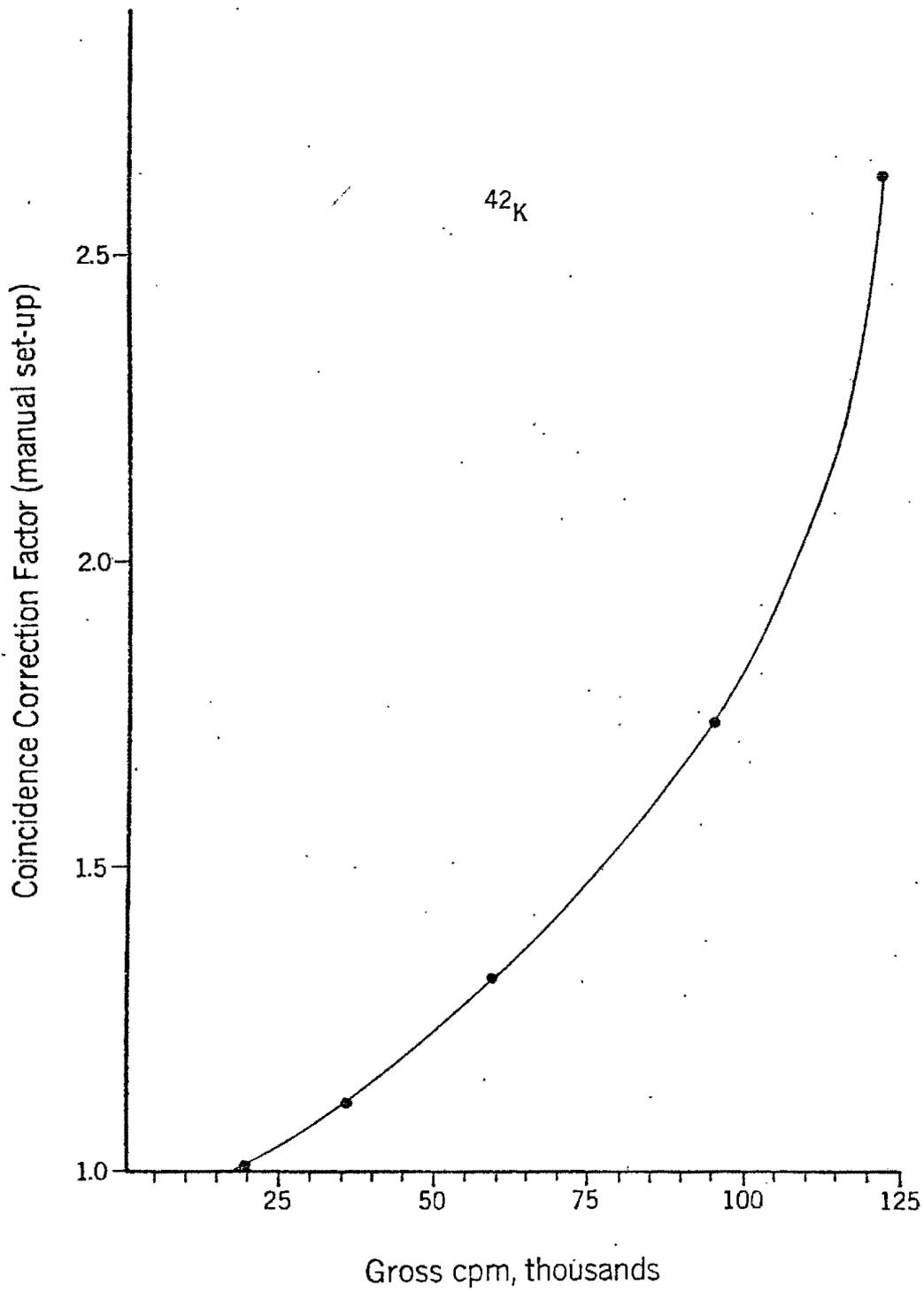
Figure 4. Coincidence correction factor versus gross cpm for ^{42}K.

SUMMARY

This work contains only the second report of net H^+ extrusion in characean cells. Net H^+ extrusion was demonstrated using a high external K^+ concentration. This phenomenon seems directly related to the amount of surplus H^+ accumulated within the cell per unit volume. That is, H^+ extrusion only occurs when the accumulated acidity per unit volume exceeds a certain value.

There is no fixed stoichiometric relationship between H^+ efflux and K^+ influx. K^+ influx always exceeds H^+ extrusion, with at least some of the charge-balancing accounted for by net Cl^- influx.

The complex nature of H^+ and K^+ transport does not lend itself to any simple correlation with observed variations in the membrane potential.

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APPENDIX

GEOMETRY CORRECTION

A special holder was designed to count radioactivity in live cells. This consisted of a section of plastic ruler mounted on a piece of plexiglass. The cell was placed on filter paper in the groove on the ruler and then the whole apparatus was placed under a GM tube. For comparison with standards a number of cells of different lengths were read live then broken up in 1.0 ml of "killing solution" (6 parts by volume 1.0 $NaHCO_3$, 1 part H_2O and 1 part ETOH) and read wet in 1 inch diameter aluminum planchettes. The standards were 1.0 ml of "killing solution" containing a known amount of the ^{42}K K solution. These were counted in the same manner as the wet cells. This enabled live cells to be corrected to wet for comparison with the standards. This correction factor, which varies with cell length may be obtained from Table 3.