Anionic Transport in *Nitella clavata*

by

Bonnie L. Bower

Submitted to the
Department of Biological Sciences
State University of New York
College at Brockport
in partial fulfillment of the
Degree of Master of Science
July 1978

THESIS DEFENSE

Bonnie L. Bower

APPROVED	NOT APPROVED	MASTER'S DEGREE ADVISORY COMMITTEE	
X		Charles E. Barn Major Advisor	July 6, 1978 Date
X		Dan Brann Committee Member	July 6, 1978 Date
X		P. Michael Fox Committee Member	July 6, 1978 Date

Joseph C. Mukerji
Chairman, Graduate Committee

Theodore J. Stout
Chairman, Dept. Biological Sciences

TABLE OF CONTENTS

INTRODUCTION

Objective

The objective of this study was to attempt to elucidate certain aspects of anion transport(specifically, Cl^-, NO_3^- and SO_4^{-2}) in Nitella clavata.

Features of Anion Transport

Anions are accumulated to very high concentrations within the vacuole, for example, the chloride concentration in the sap is approximately 100 mM(Bower, 1977, unpublished data). Such an influx occurs against an electrochemical potential gradient, and requires an input of energy. The influx of chloride ions into the cell, calculated from $G = RT \ln\frac{[Cl]_i}{[Cl]_o} + zFE_m$, requires an input of 6.18 kcal. Because an active transport mechanism appears necessary, and subsequent leakage must be prevented, permeability of the membrane to anions is low compared to that of cations.

Photosynthesis has been implicated in stimulating Cl^- influx, in that it appears that a redox process may be involved in providing the energy for Cl^- transport. Maximum Cl^- influx occurs only under conditions of net reductant formation via photosynthesis (MacRobbie, 1970). However, in far-red light, where the photosynthetic formation of ATP, but not of reductant occurs, chloride influx is reduced, at least in Hydrodictyon (Raven, 1967). This suggests that either reductant or reductant in combination with ATP is required for chloride transport. There is, however, no specific

information regarding a coupling mechanism between electron transport and chloride transport.

Raven(1968) discovered that the presence of bicarbonate inhibits Cl^- influx in Hydrodictyon. He did not find that the inhibition was due to bicarbonate being used as a carbon source, since CO_2 did not inhibit Cl^- influx(Raven, 1968). Spanswick and Miller (1977), however, found that Cl^- influx was inhibited by CO_2(in the light) from pH 5-8. This inhibition increases at lower pH. In view of these results, they have suggested that the inhibition of Cl^- does not stem from competition between Cl^- and HCO_3^- for the same carrier, and have proposed that the Cl^- inhibition is due to a decrease in the reductant necessary to drive the Cl^- transport.

MacRobbie(1969) demonstrated that Nitella transports both Br^- and Cl^-, and that Br^- can substitute for Cl^- when the latter is absent. When both are together in the suspending medium, however, Cl^- uptake is faster, and, therefore, has a smaller K_m than that for Br^-.

Hypotheses on Anion Transport

Lundegardh(1954) formulated the original theory of charge separation. He found that upon reduction of the cytochrome system, by acceptance of an electron into the iron atom of the porphyrin ring, a proton is released to the medium. He theorized that as the electrons are moved through the cytochrome system to oxygen as a final acceptor, anions are moved in a direction opposing the electrons, or inward, with cations as their ultimate acceptor.

Smith(1970) proposed a mechanism for Cl^- transport; that of a

Cl^-/OH^- countertransport(antiport) system. He presented the hypothesis that an existing pH gradient across the plasmalemma controls chloride influx. Such a pH gradient would, in turn, be dependent upon charge separation reactions with the H^+ moving outward, and OH^- inward.

Raven and Jayasuriya(1977), in their investigation of nitrate influx in Hydrodictyon africanum, have found that the assimilation of nitrate is associated with the OH^- efflux. Hydroxide ion is produced during the assimilation process, and they have proposed that the excess OH^- is disposed of by a concurrent OH^- efflux.

An alternative to Smith's Cl^-/OH^- countertransport system, would be a Cl^-/H^+ cotransport(symport) system(Spear, et. al.,1969). Such a mechanism would be energetically equivalent to a Cl^-/OH^- countertransport system(Smith and Walker,1976). Hydrogen has been identified as a participant in a hexose transport mechanism in Chlorella (Komor and Tanner, 1971). If either an anion or a hexose can cotransport with H^+, via the same carrier, a competition would arise between the hexose and Cl^-.

MacRobbie(1969,1971) has supported a vesicular mechanism for ion transport. In her mechanism, Cl^- is transported via pinocytotic vesicles(from the outside) and subsequently to the vacuole through the endoplasmic reticulum. Such a mechanism is difficult to support, however, and MacRobbie has not ruled out the possibility that the vesicles form just inside the plasmalemma, subsequent to an ion exchange across the plasmalemma. Van Steveninck(1976) has produced evidence to support MacRobbie's (1969) proposal of primary vesicular movement. After a short exposure to Cl^-, vesicular structures

which normally showed little Cl^- deposit, became greatly increased with Cl^- deposits.

Other Possible Mechanisms of Anion Influx

Recent work by Tanner(1969) and Komor and Tanner(1971) has characterized a proton-hexose cotransport system for which glucose as well as some of its analogues, such as 3-O-methylglucose and 6-deoxyglucose, act as substrates and inducers in Chlorella vulgaris. They have suggested a carrier on the outside, and a deprotonated carrier with a low affinity for sugars on the inside(Komor, et. al., 1973). When these sugars(3-O-methylglucose and 6-deoxyglucose) were added to a suspension of cells previously exposed to those same sugars, a pH shift to the alkaline side resulted. This shift did not occur with sugars which are not transported(Komor and Tanner, 1974, a, b).

Paschinger and Broda(1967) have investigated the inhibition of bromide ion active transport by glucose in Chlorella pyrenoidosa. The uptake mechanism is specific for bromide ions since excesses of other ions(excluding chloride) do not decrease bromide ion concentration in the cells. From their results, they assumed the same mechanism is involved for chloride, and so they could be interpreted in terms of chloride.

In light of Paschinger and Broda's(1967) and Komor and Tanner's work(1971), if glucose does competitively inhibit Cl^- active transport, it would be an indication that Cl^- may be cotransported with H^+ also. A Cl^-/H^+ cotransport system has previously been suggested in Spear, Barr and Barr(1969), after they observed that acid formation and chloride flux occur in the same region of the cell.

If evidence cannot be found to support glucose-anion competition for carrier sites, two alternative approaches will be used. It is possible that Nitella may not have a glucose/H^+ transport system, since it is oligotrophic, and not likely to have an environment containing much organic material, and therefore, would have no need for such a system. The approaches used will be to obtain the OH^- effluxes for Cl^- exchange first(Smith,1970), and secondly, for NO_3^- exchange, according to the recent findings by Raven and Jayasuriya(1977).

Experimental Approach

By measuring uptake of radioactive Cl^- in the presence and absence of a hexose, we will be able to determine if glucose inhibits Cl^- influx. Controlled pH studies through titration during the addition of glucose to a chloride solution will help us to determine the amount of H^+ entering the cells. If a common carrier exists for glucose and chloride, its maximum rate may be achieved for chloride alone before glucose addition, resulting in a depression of chloride influx, but not H^+ influx. Several possible results could be obtained. A glucose/H^+ transport system may not have an effect on Cl^- transport; that is, chloride could have a separate co-transport with H^+. Secondly, the presence of glucose could inhibit Cl^- without affecting H^+ competition if the cotransport system is already saturated. Thirdly, glucose may compete with Cl^- for H^+ in a cotransport system, and H^+ uptake would still be stimulated if the common carrier is not yet saturated. Finally, it is possible that a glucose/H^+ or Cl/H^+ cotransport system does not exist. Should the

results not support a Cl^-/H^+ cotransport system, the possibility of a light-driven Cl^-/OH^- countertransport system(Smith, 1970) will be investigated, along with other anions, namely NO_3^- and SO_4^{-2}.

Studies on NO_3^- and SO_4^{-2} are of an exploratory nature and may form the basis of future studies.

A recent study made by Raven and Jayasuriya(1977) suggested a NO_3^-/OH^- exchange with a stoichiometry of 1, in Hydrodictyon. Although this result could be interpreted equally as well in terms of a $NO3^-/H^+$ cotransport, the problem will be treated as according to Raven and Jayasuriya(1977).

In such experiments, however, certain problems arise in controlling the conditions. Concurrent H^+ extrusion would complicate assesment of the experimental results. Therefore, K^+ must be eliminated in order to prevent a H^+/K^+ exchange(Barr, Holland and Bower,1977) at a pH of 5.7. It is also necessary to determine if the mechanism is specific, or if chloride or sulfate can substitute.

MATERIALS AND METHODS

Nitella clavata, a member of the Characeae class, having giant internodal cells, was used for the experiments. The algal plants were grown in open tanks of culture solution(Table 1), under 16 hours of light from fluorescent lamps(one-half Gro-lux, and one-half cool white), alternating with 8 hours of darkness. All cells used in experiments up to exp. 32 were grown in solutions containing 0.3 mM Tris(hydroxymethyl)aminomethane. For experiments 32 through 37, the cells were collected from culture containing no Tris.

The light intensity was 100 foot-candles at the surface of the cultures.

Cells were harvested by clipping away neighboring cells with dissecting scissors. Only the first, second or third internodal cells proximal to the apical tip were used. They ranged from 2.5 to 5.0 cm in length, and 600 to 1,000 um in diameter. Harvested cells were then conditioned from one to seven days at 22° C. under 37 foor-candles of cool-white fluorescent light. The solution normally used(except where another is designated)was K solution, consisting of (expressed in mmoles/liter): 1.0 KCl, 0.1 NaCl, 0.1 $CaCl_2$ and 0.1 $MgCl_2$ at a pH of 5.7 or 7.0, neutralized with KOH.

Resting Membrane Potentials

In order to obtain membrane potential measurements, a single cell was placed in a narrow plexiglass trough. The solutions to be used, flowed by gravity through the trough at a rate of about 2 ml/minute, through polyethylene tubing connected to bottles containing the experimental solutions.

Glass microcapillary electrodes with tip diameters of 5 to 10um, were made with a David Kopf 700C Vertical Pipette Puller; a chlorided silver wire was inserted into each capillary tube. The reference electrode consisted of a pH type reference reservoir, also with a chlorided silver wire in it. Both were filled with a solution simulating the Nitella vacuolar sap: 80 mM KCl, 20 mM NaCl, and 5 mM $CaCl_2$. The open end of the microcapillary was covered with silicone grease to prevent evaporation of the filling solution.

The microelectrodes were held in place and moved by means of a Narishige Micromanipulator. The microelectrodes were then inserted

into the vacuole through the cell wall, plasmalemma and tonoplast, with the aid of a Titan 1218 dissecting scope. Measurements were made on a Keithley 604 Differential Electrometer Amplifier and recorded on a Leeds-Northrup Speedomax Recorder.

A measurement of the electrical potential contributed by the microelectrode itself was accomplished by immersing the tip into the same solution as was used to fill the microelectrode; this was connected electrically to the solution running through the trough by a filter paper bridge. This potential was measured before and after each experiment, and only those microelectrodes with tip potentials of $\pm$15 mV or less were used.

H^+ Influx and OH^- Efflux

Net hydrogen ion influxes and hydroxide effluxes were measured by titration. Eighteen to fifty cells were placed in 15 to 30 ml of solution into which a pH probe was immersed. Changes in pH were detected with either a Leeds-Northrup 7410 or Corning 10 pH meter. The solution was titrated in order to maintain the pH approximately at its starting value by using a 5 ml buret containing 5mM or 10mM HCl. The solutions were mixed using a 10 ml pipet to assure an even dispersal of hydrogen and hydroxide ions, and thus, a true pH reading.

Unidirectional Chloride Influx

Radioactive chloride in the form of $K^{36}Cl$ with a specific activity of 5.8 mCurie/mmole Cl was used. For unidirectional chloride influx measurements, cells conditioned for 7 days were placed in K solution or in the appropriate experimental solution(Table 2), con-

taining ^{36}Cl, for 3 to 15 hours.

After the ^{36}Cl absorption period, a four phase, 90 minute rinse in a non tracer solution or the appropriate experimental solution (Table 2) followed. Radioactivity was then determined by placing each cell, in turn, in a specially constructed holder which slid beneath a Geiger-Muller tube connected to a Nuclear-Chicago 8703 Decade Scaler. The reading for each cell was subsequently corrected for geometry and self-absorption(Ryan,1973).

Unidirectional Chloride Efflux

Unidirectional chloride efflux was measured by placing each cell in 5 ml of nontracer solution for 48 hours. At the conclusion of this period, 1 ml of this solution was removed, placed in a planchet along with one drop 1 M $NaHCO_3$, and then dried on a hot plate. Radioassay was made with a Nuclear-Chicago low background gas flow counter.

In order to determine the specific activity of the intracellular chloride, it was necessary to measure the vacuolar sap chloride concentration. This was accomplished by piercing the cell wall with a needle, and collecting the vacuolar sap in a 5 ul capillary pipet and then delivering it onto a paraffin block. The concentration was then measured potentiometrically by using a chlorided silver wire and reference microelectrode. The latter was the reference electrode. of small pH combination electrode(Model MI-410, Microelectrode, Inc.).

RESULTS

Chloride Uptake

Some preliminary experiments were carried out to determine the solution composition which would give the greatest Cl^- uptake. Table 3, and Figure1 show that Cl^- uptake was not substantially improved in the presence of phosphate, contrary to a previous result (DiGregorio, unpublished data). It was also determined that the labelled Cl^- efflux was so very small(0.1% of the influx) that it would not significantly effect the value of the Cl^- readings. Because the fastest Cl^- uptake was observed in K solution, it was used for the remainder of the chloride uptake experiments.

H^+/Hexose Cotransport and its Relationship to Cl^-

Table 4 and Figures 2 and 3 show the various combinations used to determine which would provide the maximum H^+ influx. At this point, a switch was made to cells grown in cultures without Tris, since cells grown in Tris have rather high concentrations of NH_4^+ in the vacuole. The loss of NH_3 by cells would tend to complicate the measurement of H^+ influx since both NH_3 efflux and H^+ influx result in an increase in external alkalinity. In Exp. 19, the change in concentration from 10 to 20 mM galactose made little difference in H^+ influx, nor did alkaline pretreatment. The H^+ influx in K solution actually was, on the whole, slightly higher than with the addition of a hexose. In Exps. 23 and 24, it can be seen that a more acid pH of 5.0 resulted in greater fluxes, possibly due to the greater availability of H^+. Again, there was little difference in the fluxes between K and K plus hexose, or as a result of an eight- fold

increase in concentration of hexose(from 20 mM to 160 mM). Hydrogen influxes, however, seemed slightly higher with the higher concentrations, so 160 mM hexose was used for the radioisotope tracing of $^{36}Cl^-$ influx.

Chloride uptake(Table 5) in the presence of glucose or galactose was measured and compared to a control(K solution). The mean values for ^{36}Cl uptake for three groups of 15 cells each showed no significant difference at the p=0.05 level between the control(K) and K plus glucose or galactose.

Membrane potentials were measured in a series of solutions progressing from K solution to K+160 mM glucose and back to K solution. As shown in Table 6, upon introduction of 160 mM glucose to the bathing solution, the cells exhibited a mean depolarization of +56 mV, which is significant at the p=0.01 level.

NO_3^- and SO_4^{-2} Transport

In experiments 32-37, an attempt was made to induce high net OH^- effluxes, in order to test Smith's(1970) Cl^-/OH^- hypothesis. Table 7 shows the cumulative OH^- production in 90 minutes under varying conditions of light, pretreatment and solution composition.

The increase in light from 42 Fc to 350 Fc for 2 mM NO_3^- solutions in Exps. 32,33 and 34 seemed to produce an increase inOH^- production. The increase from 300 Fc to 600 Fc, however, made little difference in the OH^- production. Pretreatment did not appear to have a large effect on OH^- efflux results with 2 mM NO_3^- present. Conditioning at 6 days K, pH 5.7, followed by 2 days K, pH 7.0 did not produce very different values from conditioning at 7 days K, pH 7.0.

Contrary to Raven and Jayasuriya's results on Hydrodictyon(1977), changes in nitrate concentration from 0.5 mM to 2.5 mM, produced little variation in total OH^- production. The same was true for changes in sulfate concentration, nor did combinations of different molarities of NO_3^-, SO_4^{-2} and Cl^- seem to change the cumulative OH^- production (Figures 4 and 5). Addition of 1 mM NO_3^- or 1 mM SO_4^{-2} or both resulted in values for total hydroxide release which were too close to be separated(Fig.4). Figure 5 shows that OH^- release was slightly dependent on whether Cl^- or NO_3^- was the anion present. After the first half hour, the net OH^- efflux was very similar, regardless of the presence or absence of K^+.

Membrane potentials were obtained on cells in solution containing 1 mM SO_4^{-2} and 2 mM NO_3^- and compared to K solution(1.5 mM Cl^-) in order to observe any electrical effects of the anions. Table 8 shows that the membrane potentials exhibited a significant hyperpolarization in the presence of SO_4^{-2} and NO_3^-(-19 mV and -24 mV respectively) at 600 Fc. It is likely that the effect is due partially to the presence of a cation, in this case, Ca^{+2}. No $CaCl_2$ control was used, therefore, no firm conclusion can be arrived at because of the two variables.

DISCUSSION

Cl^-/H^+ Cotransport

We could find no evidence to support the hypothesis that Cl^- cotransports with H^+. Contrary to Komor and Tanner's(1971) report of a well characterized hexose/H^+ cotransport system in Chlorella vulgaris, we found no evidence for the existence of such a system in Nitella:

H^+ influx did not increase as compared to the control(K solution) when hexose was added. It had been thought that if both glucose and chloride were cotransported with H^+, glucose might competitively inhibit Cl^- transport. The inhibition of Cl^-(andBr^-) by hexoses had previously been demonstrated by Paschinger and Broda(1967) in Chlorella pyrenoidosa, before Komor and Tanner's(1971) work on the hexose/H^+ system.

Radioisotope tracing of $^{36}Cl^-$ in cells both in the presence and absence of a hexose made no significant difference in the chloride uptake, nor did the nature of the hexose itself(glucose or galactose) make a difference. The only conclusion that can be drawn from these experiments is that a hexose/H^+ cotransport system does not necessarily contradict Paschinger and Broda(1967), since we used an organism which may not have a mechanism to transport hexoses.

The membrane potential of Nitella in K solution + 160 mM glucose showed a significant depolarization(+56 mV) as compared to K solution. Komor and Tanner(1977) found the membrane potential of Chlorella vulgaris depolarized upon addition of a hexose, and presented it as evidence of an electrogenic transport system. Andrianov and Kurella (1963), however, working with Nitella flexilis, found that the membrane depolarized upon addition of sucrose to the system, the depolarizations being larger with increasing molar concentrations of sucrose. They have attributed the change to the increase in tonicity of the bathing solution resulting from the addition of a nonelectrolyte(sucrose) which causes an outflow of cellular water. The release of water pressure, in turn, will cause an increase in the intracellular concentrations of other ions, such as K^+. This would provide an adequate explanation for my results, since there

was no evidence for glucose transport. Water release was probably insignificant compared to the reduction in hydrostatic pressure which probably took place..

Nitrate and Sulfate Transport

From the results of these experiments, we were unable to find conclusive evidence for a NO_3^-/OH^- countertransport system. Such evidence has been found in three other systems by Raven and Jayasuriya(1977) in Hydrodictyon africanum, Ullrich and Eisele(1977) in Ankistrodesmus braunii and Loppert, Kronberger and Kandeler (1977) in Lemna paucicostata 6746. Both Raven and Jayasuriya(1977) and Ullrich and Eisele(1977) found their systems to have a stoichiometry of 1.0. Ullrich and Eisele(1977) did not find any evidence for involvement of a cation for charge balance, and so, based upon alkalinisation of the medium when NO_3^- is added, suggested OH^- was involved in countertransport.

One problem is to search for what balances such a large OH^- efflux. James P. Zorn(unpublished data) has found that under bright light conditions(600 Fc, cool-white), the Cl^- influx is still very small, despite a high OH^- efflux. Loppert, et. al.(1977) discovered that in the absence of NO_3^-, K^+efflux balances the OH^- efflux. In the presence of external NO_3^-, then, NO_3^- influx might balance OH^- efflux.

The high OH^- effluxes obtained in bright light by both Zorn (unpublished data) and myself seem to reflect an involvement of light in driving the transport of at least this ion. In the case of Cl^- transport, Takeda and Senda(1974), found Cl^- influx to be

stimulated 10 to 20 fold in the light, although such stimulation occurred only in that part of the cell which was illuminated.

We, however, did not see any greater alkalinisation than that with control(Cl^-) solutions, irrespective of anion added, even under varying concentrations. No anion fluxes were measured other than those of OH^-.

The hyperpolarization of the membrane potential upon addition of SO_4^{-2} and NO_3^- has previously been shown to be due to the presence of Ca^{+2}(Rent, 1971) and certainly contributed to, by the absence of K^+, rather than to SO_4^{-2} or NO_3^-.

SUMMARY

Experimentation provided no support for the hypothesis that Nitella clavata possesses a hexose/H^+ cotransport system. We were, therefore, unable to provide evidence for a Cl^-/H^+ cotransport system through competitive inhibition with a hexose/H^+ mechanism.

The study of anionic transfer was basically of an exploratory nature, but preliminary results do not seem to support a NO_3^-/OH^- countertransport system. For more conclusive results, more experiments would be needed, such as radioisotope tracing of NO_3^-, Cl^- and SO_4^{-2} in the presence of other anions.

TABLE I

Composition of culture solution IV-47

Concentration (mM)	Material
0.4	KNO_3
1.0	$MgSO_4$
1.0	NaCl
1.0	$NaHCO_3$
0.3	Tris·HCl
1.5	$CaCl_2$
0.01	Na_2MoO_4
0.037	H_3BO_3
0.01	KH_2PO_4
5 ppm	streptomycin
0.5 ml/liter	micronutrient solution IV-35

pH adjusted to 7.5 - 8.0 with 1.0 M NaOH
Composition of micronutrient solution IV-35 :
14g humus soil boiled for 1 hour in 200 ml of 10 mM nitrilotriacetic acid neutralized to pH 9.2
Final pH 8.5.

TABLE II

Composition Of Experimental Solutions

Solution K-less K	Solution K
0.1 mM Nacl	1.0 mM KCl
0.1 mM $CaCl_2$	0.1 Mm NaCl
0.1 mM $MgCl_2$	0.1 mM $CaCl_2$
	0.1 mM $MgCl_2$

Solution H75-65	Solution B75-74
1mM KCl	0.5 mM KCl
0.1 mM $CaCl_2$	0.1 mM $CaCl_2$
0.1 mM $MgCl_2$	0.1 mM $MgCl_2$
0.1 mM $Na_{1.05}H_{1.95}PO_4$	0.1 mM NaCl
	0.5 mM $K_{1.05}H_{1.95}PO_4$

TABLE III

The difference in Cl^- uptake with variation in phosphate concentration in external solution. All cells were conditioned at 22° C, 37 fc at a pH of 5.7.

Exp.	Cell Age	Experimental And Conditioning	^{36}Cl Uptake ($\times 10^{-8}$ mol/cell)	^{36}Cl Influx (pmol/cm^2 sec)	Flux Period (hrs)
9A	8	K	0.075	0.02	0-17
	8	H 75-65	0.07	0.02	0-17.5
10A	8	K	0.632	0.14	0-17
	8	H 75-65	0.784	0.17	0-19
13A	7	K	2.32	0.598	0-15
	7	H 75-65	3.84	0.64	0-15
	7	B 75-74	3.60	0.69	0-16.7
13B	8	K	4.42	0.50	15-35
	8	H 75-65	5.10	0.40	15-33.5
	8	B 75-74	5.85	0.53	17-34
13C	10	K	10.57	0.54	35-79
	10	H 75-65	12.39	0.42	35.5-78.2
	10	B 74-75	9.47	0.23	34-78
			^{36}Cl Released ($\times 10^{-8}$ mol/cell)	^{36}Cl Efflux (pmol/cm^2 sec)	
13E	15	K	0.53	0.046	48
	15	H 75-65	0.39	0.021	48
	15	B 74-75	0.35	0.026	48

TABLE IV

Hydrogen influx in the presence of a hexose. All cells were grown in Tris and conditioned at 22° C, 37 fc. All Exp. 19 was calculated from pH assuming no buffering. Exp. 19 was at a pH of 5.7; Exp. 23 and 24 at pH 5.0. The uptake time for Exp. 23 and 24 is 90 minutes.

Exp.	Cell Age (days)	Experimental Solution	Uptake ($\times 10^{-8}$ mol/cell)	Influx (pmol/cm^2 sec)	Saturation Time (min)
19D	1	K	0.032	1.53	33
		K+ 20 mM galactose	0.032	1.07	17
E	1	K	0.04	1.15	7
		K+ 10 mM galactose	0.06	1.00	10
		K	0.03	0.86	7
F	1	K	0.08	2.65	80
		K+ 20 mM galactose	0.03	1.14	7
		K	0.02	0.96	20
G	1	K alkaline (pretreat) 1 night	0.06	0.87	70
		K+ 20 mM galactose	0.05	2.5	9
		K	0.03	0.70	15
H	1	K	0.04	2.01	10
		k+ 20 mM galactose	0.03	0.64	40
		K	0.02	0.91	10
I	2	K alkaline (pretreat) 2 nights	1.0	1.72	135
		K+ 20 mM galactose	0.02	0.93	10
		K	0.01	0.42	20
23A	7	K	1.66	3.66	none
		k+ 20 mM galactose	0.18	1.97	18
B	7	K	2.6	13.4	70
		K+ 20 mM galactose	3.0	15.2	50
24	7	K	2.88	18.9	none
		K+ 160 mM glucose	2.50	33.3	none
		k+ 160 mM galactose	1.53	16.29	none

TABLE V

All conditioning was in K solution for 7 days at 22° C, 37 fc, and experimental conditions at pH 5.7, 42 fc and in the experimental solutions for 3 hours.

Exp.	Experimental Solutions	^{36}Cl Uptake ($\times 10^{-8}$ moi/cell)	^{36}Cl Influx (pmol/cm^2sec)	Std. Dev. $\Delta\bar{X}$(K-K+ Hexose)	Std. Error
25	K	0.306	0.30		
	K+ 160 mM glucose	0.235	0.26	±2.69	0.696
	K+ 160 mM galactose	0.273	0.22	±3.07	0.794

TABLE VI

Exp.	Cell Age (days)	Membrane Potentials (mv) K Solution	K+ 160mM glucose	K Solution	ΔE m(mv)
29A	1	-143	-88	-144	+56
B	5	-117	-77	-125	+44
C	6	-155	-97	-137	+49
D	5	-148	-95	-145	+51
E	7	-106	-41	-132	+78
				Mean	+56
				Std. Dev.	±13.6
				S. E.	5.92**

** Figures are significantly different at the p = 0.01 level.

TABLE VII

All cells were cultured in solution without Tris, and experiments were at pH 7.0 except 32 A1 (pH 5.7), 37 fc and 22° C.

Exp.	Cell Age (days)	Lighting (fc)	Conditioning	Experimental Soln. mM Ions	Cum. OH^- Prod. 90min. ($\times 10^{-8}$ mol/cell)	Av SA/cell (cm^2)
32A1	6	42	K soln. pH 5.7	0.1 Ca, Na, Mg 0.5 Cl 2.0 NO_3	0.828	1.20
2	8	350	2 da. K, pH 7.0	same	4.33.	1.20
B	8	42	6 da. K, pH 5.7 2 da. K, pH 7.0	same	0.766	1.13
		350		same	1.60	1.13
33	7	350	6 da. K, pH 5.7 1 da. K, pH 7.0	K soln.	1.49	1.08
			followed by	0.1 Ca, Na, Mg 0.5 Cl 2.0 NO_3	5.48	
	8	350	6 da. K, pH 7.0	same	9.77	1.08
34	5	350	5 da. K, pH 7.0	0.1 Mg, Na 1.1 Ca 1.5 Cl 1.0 NO_3	9.40	0.992
36	7	600	7da. K, pH 7.0	0.1 Mg, Na 1.1 Ca 1.0 SO_4 0.5 Cl	10.4	1.06
	7	600	same	0.1 Mg, Na 1.1 Ca 0.5 Cl, NO_3 0.75 SO_4	10.26	1.06
	7	600	same	0.1 Mg, Na 1.1 Ca 0.5 Cl 2.0 NO_3	10.8	1.06
37	7	600	3da. K, pH 7.0	(2.5 Cl)		
			4da. 1.67xK, pH7.0	1.67xK	7.96	0.913
	7	600	same	0.1 Mg, Na 1.1 Ca 2.5 Cl	5.3	0.913
	7	600	same	0.1 Mg, Na 1.1 Ca 2.5 NO_3	8.37	0.913

TABLE VIII

Membrane potentials

All cells were run at pH 7.0, 37 fc.

Exp.	Cell Age (days)	Lighting (fc)	K	K-less K+ 1mM $CaSO_4$	K-less K+ 1mM $Ca(NO_3)_2$	K	$\Delta_{1,2}$	$\Delta_{1,3}$
35A	5	42	-102	-181	-189	-114	-73	-81
B	6	42	-107	-152	-160	-102	-48	-56
C	7	600	-99	-129	-133	-98	-31	-35
D	8	600	-108	-135	-138	-120	-21	-24
E	5	600	-113	-132	-140	-123	-14	-22
F	7	600	-122	-151	-149	-125	-27	-25
G	8	600	-125	-146	-156	-160	-4	-14
	Mean (at 600 fc)		-113	-139	-143	-125	-19**	-24**
	Std. Dev.		± 10.5	± 9.4	± 9.2	± 22	± 10.7	± 7.5
	S. E.		4.7	4.2	4.1	10	4.8	3.36

** Significantly different at the p = 0.01 level.

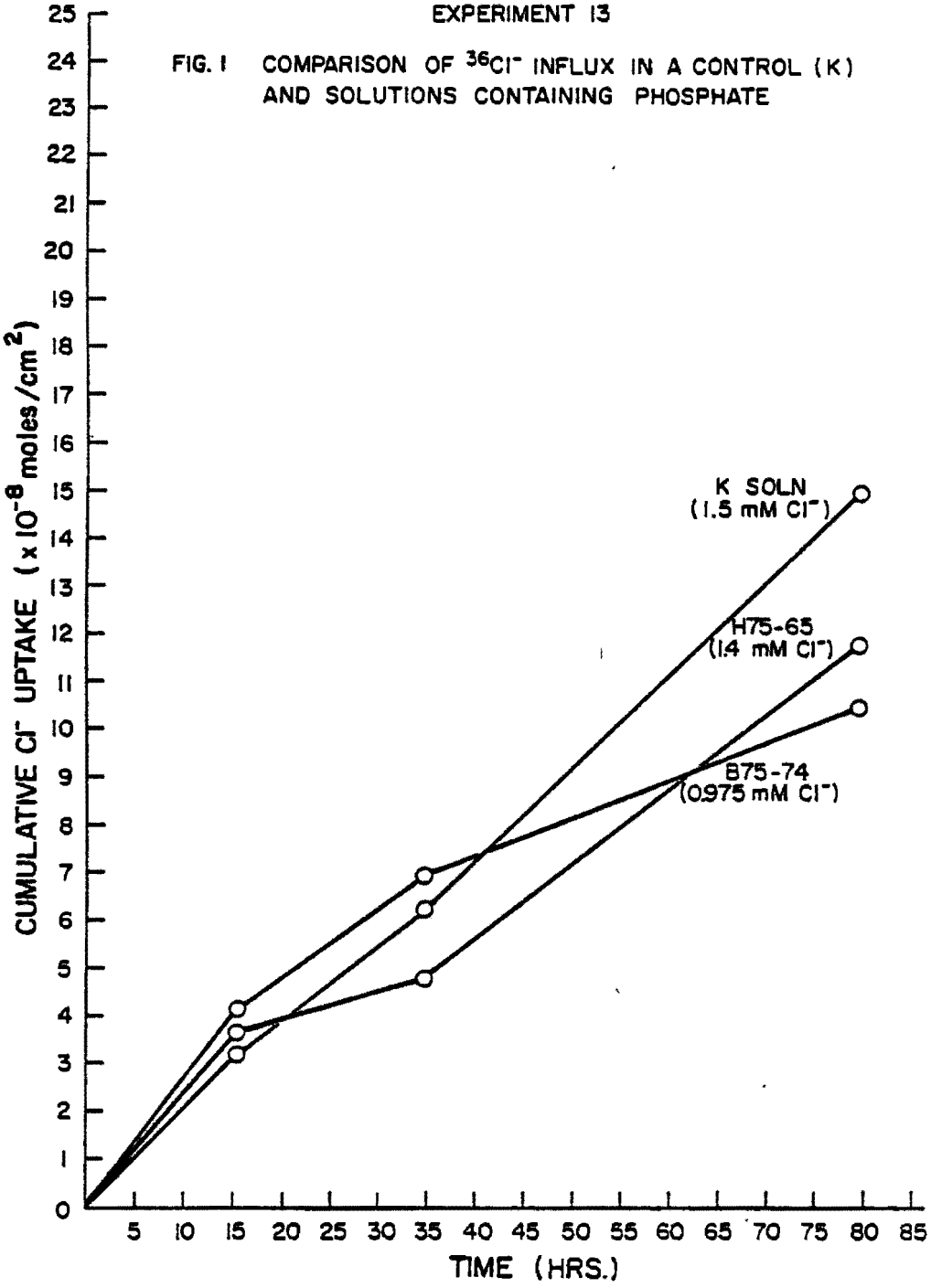

FIG. 1 See Table III for individual Cl^- flux figures. The mean fluxes ($\times 10^{-12}$ mol / cm^2 Sec.) are: 0.55 for K, 0.49 for H75-65 and 0.48 for B75-74.

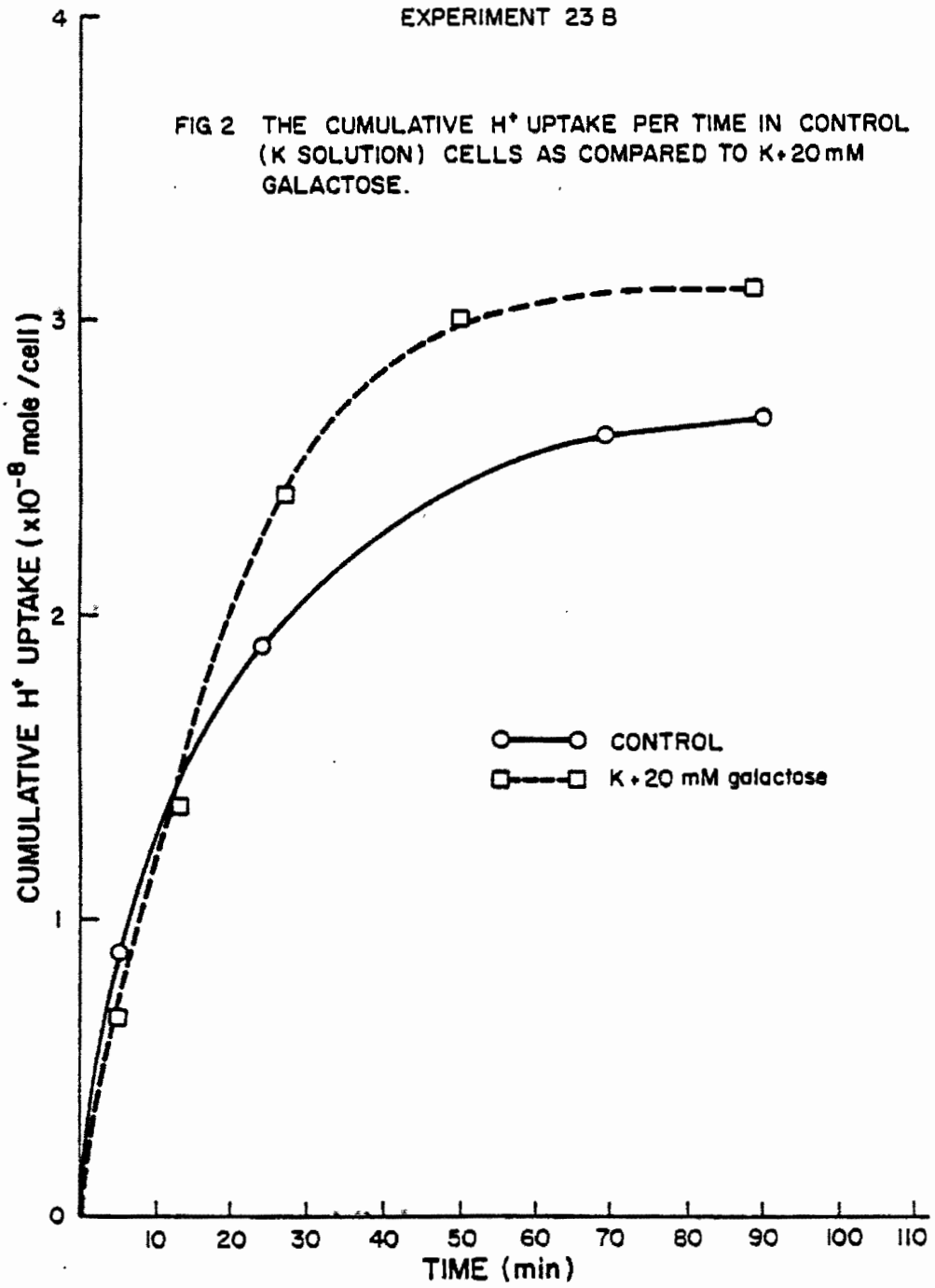

FIG. 2 See Table IV for H^+ influx figures. For 23 cells, the initial flux in K solution was 0.38×10^{-10} mol / cm^2 and for K+20 mM galactose, was 0.32×10^{-10} mol / cm^2 Sec.

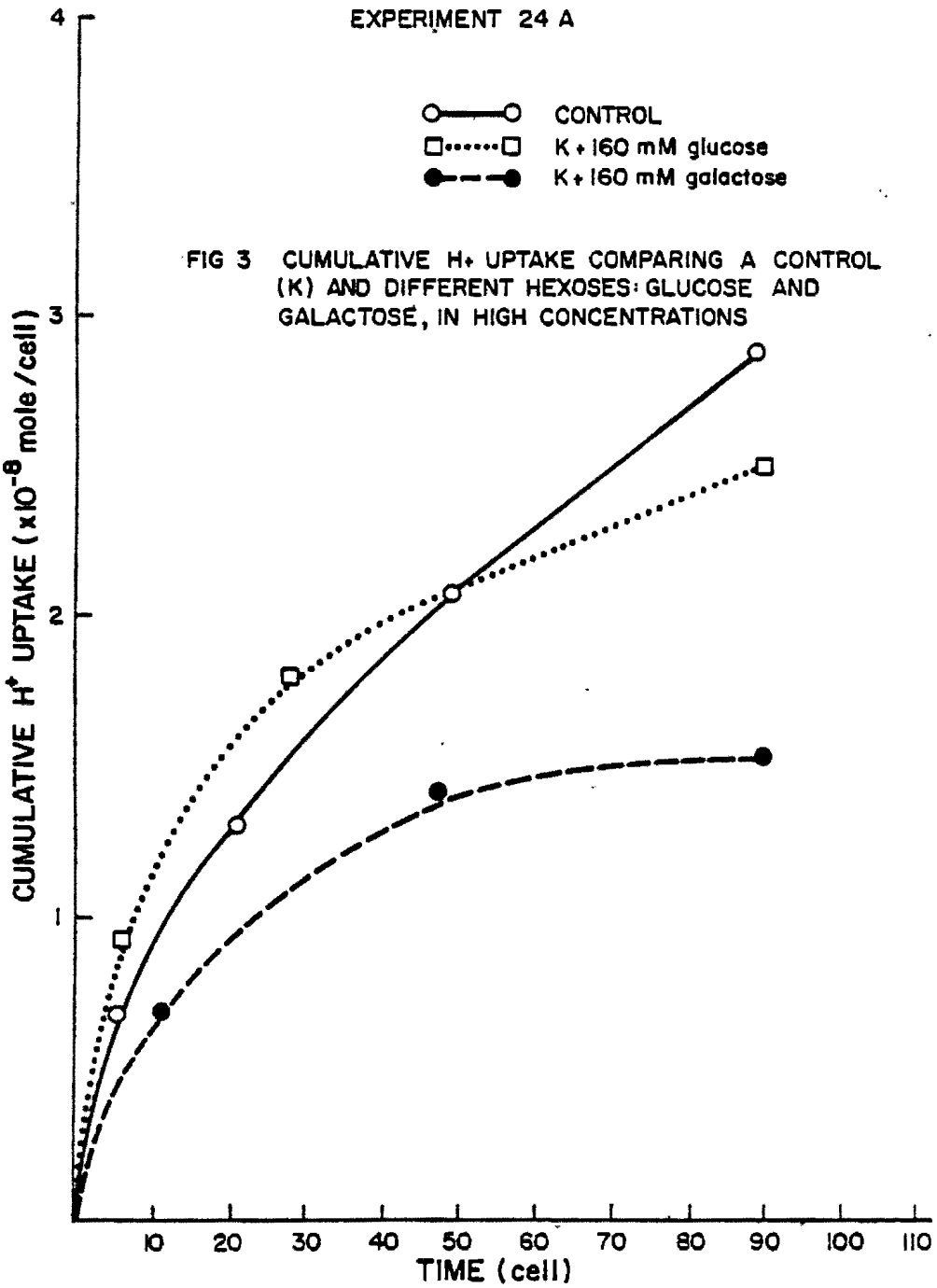

FIG 3 CUMULATIVE H+ UPTAKE COMPARING A CONTROL (K) AND DIFFERENT HEXOSES: GLUCOSE AND GALACTOSE, IN HIGH CONCENTRATIONS

FIG. 3 See Table IV for H^+ influx figures. For 18 cells, the initial flux (X 10^{-10} mol / cm^2 Sec.) in K Solution was 0.35, 0.93 in K+ 160 mM glucose and 0.33 in K+160 mM galactose.

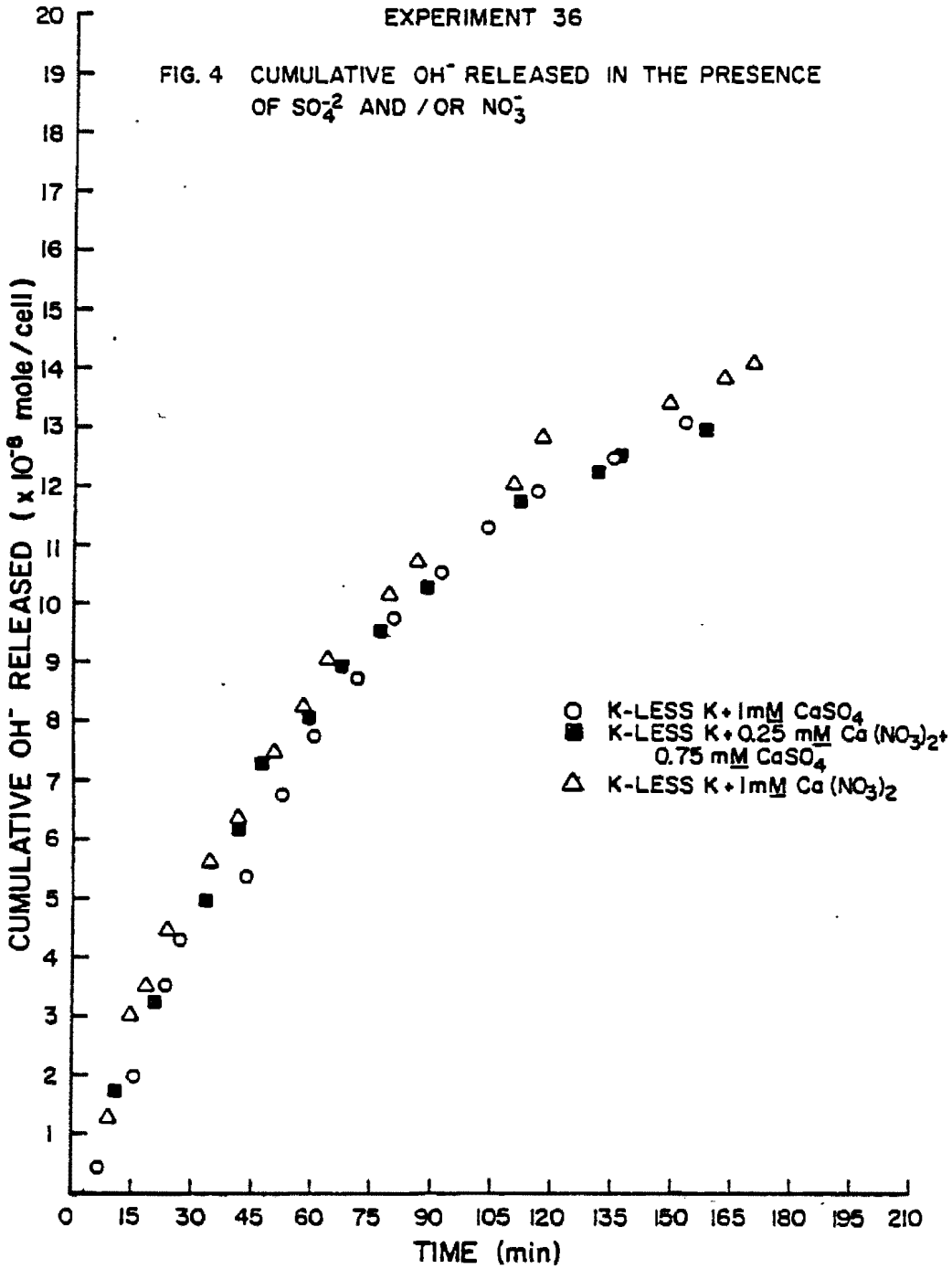

FIG. 4 The OH released per unit surface area was in terms of (10^{-8} mole / 26.50 cm^2) for 25 cells. K-less K solution contains 0.1 mM each of NaCl, $CaCl_2$ and $MgCl_2$.

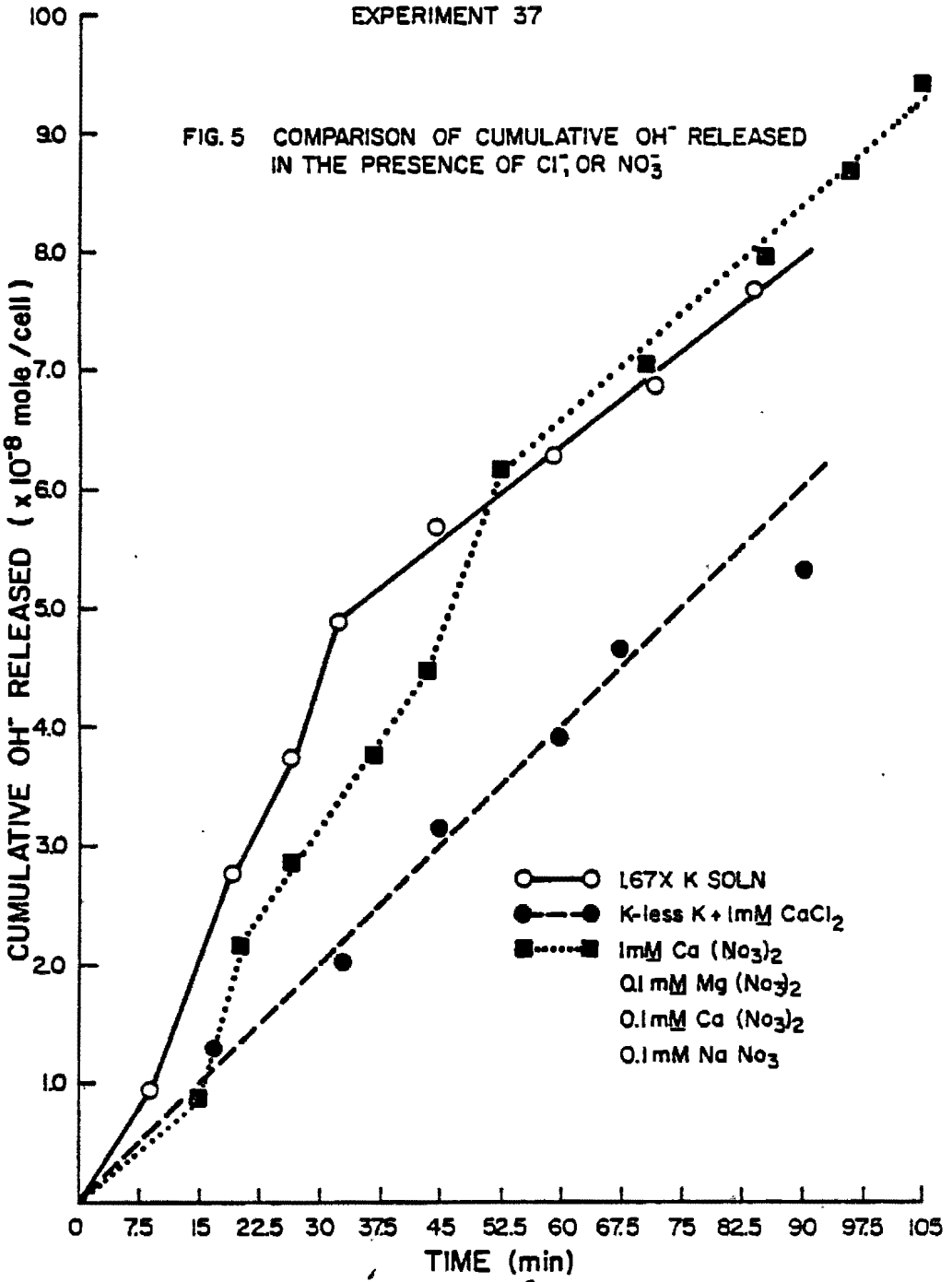

FIG. 5 The mean OH efflux (pmol / cm^2 Sec.) for 25 cells in the solution with only NO_3^- was 0.97, 0.72 for K - less K + $CaCl_2$ and 1.1 for 1.67 x K solution. The OH^- efflux per unit surface area was (X 10^{-8} mol / 13.8 cm^2). K - less K solution contains 0.1 mM each of NaCl, $CaCl_2$ and $MgCl_2$.

REFERENCES

Andrianov, V. K. and G. A. Kurella. 1963. Investigation of the nature of the resting potential of Nitella cells-1. Dependence of the resting potential of the concentration of potassium ions in the medium and its osmotic pressure. Biofizika 8:457.

Barr, C. E., D. J. Holland and B. L. Bower. 1977. Promotion of H^+ extrusion in Nitella by external K^+. In: Transmembrane Ionic Exchanges in Plants, M. Thellier, A. Monnier, M. DeMarty and J. Dainty, eds. CNRS, Paris and the University of Rouen. pp. 179-184.

Goldman, D. E. 1943. Potential impedance and rectification in membranes. J. Gen. Physiol. 27: 37.

Kitasato, H. 1968. The influence of H^+ on the membrane potential and ion fluxes of Nitella. J. Gen. Physiol. 52: 60.

Kömör, E. and W. Tanner. 1971. Characterization of the active hexose transport system of Chlorella vulgaris. Biochim. Biophys. Acta. 241: 170.

Komor, E., et. al. 1973. The active hexose uptake system of Chlorella vulgaris. K_m values for a 6-deoxyglucose influx and efflux and their contributions to sugar accumulation. Eur. J. Biochem. 39: 193.

Komor, E. and W. Tanner. 1974a. Proton movement associated with hexose transport in Chlorella vulgaris. In: Membrane Transport in Plants. U. Zimmerman and J. Dainty, eds. Springer-Verlag, Berlin-Heidelberg. p. 209.

Komor, E. and W. Tanner. 1974b. The hexose-proton cotransport system of Chlorella. J. Gen. Physiol. 64: 568.

Komor, E. and W. Tanner. 1977. Transport of hexoses depolarizes the membrane potential of Chlorella cells. In: Transmembrane Ionic Exchanges in Plants. M. Thellier, A. Monnier, M. De Marty and J. Dainty, Eds. CNRS, Paris and the University of Rouen. pp. 415-421.

Loppert, H. G., W. Kronberger and R. Kandeler. 1977. Correlation between nitrate uptake and alkalinisation by Lemna paucicostata 6746. In: Transmembrane Ionic Exchanges in Plants. M. Thellier, a. Monnier, M. De Marty and J. Dainty, eds. CNRS, Paris and the University of Rouen. pp. 283-288.

Lundegardh, H. 1954. Anion respiration: the experimental basis of a theory of absorption, transport and exudation of electrolytes by living cells and tissues. Sym. Soc. for Exper. Biol. 8:262.

MacRobbie, E. A. C. 1969. Ion fluxes to the vacuole of Nitella translucens. J. Exp. Bot. 20:236.

MacRobbie, E. A. C. 1970. The active transport of ions in plant cells. Q. Rev. Biophys. 3:251.

MacRobbie, E. A. C. 1971. Vacuolar fluxes chloride and bromide in Nitella translucens. J. Exp. Bot. 22:487.

Paschinger, H. and E. Broda. 1967. Hemmung der acktiven Bromidaufnahme durch Pflanzenzellen durch Glucose. Monatshefte fur Chemie. 98:905.

Raven, J. A. 1967. Light stimulation of active ion transport in Hydrodictyon africanum. J. Gen. Physiol. 50:1627.

Raven, J. A. 1968. The linkage of light-stimulated Cl^- influx to K and Na influxes in Hydrodictyon africanum. J. Exp. Bot. 19:233.

Raven, J. A. and H. D. Jayasuriya. 1977. Active nitrate assimilation and hydroxyl ion efflux by Hydrodictyon africanum. In: Transmembrane Ionic Exchanges in Plants. M. Thellier, A. Monnier, M. DeMarty and J. Dainty, eds. CNRS, Paris and the University of Rouen. pp.299-305.

Rent, R. 1971. Masters Degree Thesis. SUNY College at Brockport, Brockport, New York.

Ryan, T. 1973. Masters Degree Thesis. SUNY College at Brockport, Brockport, New York.

Smith, F. A. 1970. The mechanism of chloride transport in Characean cells. New Phytol. 69:903.

Smith, F. A. and N. A. Walker. 1976. Chloride transport in Chara corallina and electrochemical potential difference for hydrogen. J. Exp. Bot. 27:451.

Spanswick, R. M. and A. C. Miller. 1977. The effect of CO_2 on the Cl^- influx and electrogenic pump in Nitella translucens. In: Transmembrane Ionic Exchanges in Plants. M. Thellier, A. Monnier, M. DeMarty and J. Dainty, eds. CNRS, Paris and the University of Rouen. pp. 239-245.

Spear, D. G., J. K, Barr and C. E. Barr. 1969. Localization of hydrogen ion and chloride ion fluxes in Nitella. J. Gen. Physiol. 54:397

Takeda, J. and M. Senda. 1974. Effect of light on sodium and chloride influxes in partly illuminated Nitella flexilis cells. Plant and Cell Physiol. 15: 957.

Tanner, W. 1969. Light-driven active uptake of 3-O-methylglucose via an inducible hexose uptake system of Chlorella. Biochem. Biophys. Res Commun. 36: 278.

Ullrich, W. R. and R. Eisele. 1977. Relations between nitrate uptake and nitrate reduction in Ankistrodesmus braunii. In: Transmembrane Ionic Exchanges in Plants, M. Thellier, A. Monnier, M. De Marty and J. Dainty, eds. CNRS, Paris and the University of Rouen. pp.307-313.

Van Steveninck, R. F. M., M. E. Van Steveninck, P. O. Peters, and T. A. Hall. 1976. Ultrastructural localization of ions. J. Exp. Bot. 27:1291.