WATER AND ION EF-flux IN
ISOLATED ONION ROOTS

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WATER AND ION EFFLUX IN
ISOLATED ONION ROOTS

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By

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>List of Tables</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>iv</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>List of Illustrations</th>
<th>v</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF ILLUSTRATIONS</td>
<td></td>
</tr>
</tbody>
</table>

## Chapter

### I. INTRODUCTION

1

### II. METHODS AND PROCEDURES

10

- General Methods
- Inhibitor Solutions
- Radioisotope Solutions
- Experimental Procedure

### III. RESULTS

19

- General Control Experiments
- Water and Na\textsuperscript{22} Efflux
- Water and Rb\textsuperscript{86} Efflux

### IV. DISCUSSION

34

### V. SUMMARY

46

### BIBLIOGRAPHY

48
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. A Summary of the Effects of Azide and Dinitrophenol of Water and Rb(^{86}) Efflux in Isolated Onion Roots</td>
<td>33</td>
</tr>
</tbody>
</table>

iv
## LIST OF ILLUSTRATIONS

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-A.</td>
<td>Micro-Potometric Apparatus</td>
<td>14</td>
</tr>
<tr>
<td>1-B.</td>
<td>Micro-Potometer in Humidity Chamber</td>
<td>14</td>
</tr>
<tr>
<td>2.</td>
<td>Water Efflux from Isolated Onion Roots</td>
<td>21</td>
</tr>
<tr>
<td>3.</td>
<td>Potassium Efflux from Isolated Onion Roots</td>
<td>22</td>
</tr>
<tr>
<td>4.</td>
<td>Azide Effects on Water and Na(^{22}) Efflux</td>
<td>24</td>
</tr>
<tr>
<td>5.</td>
<td>DNP Effects on Water and Na(^{22}) Efflux</td>
<td>24</td>
</tr>
<tr>
<td>6.</td>
<td>Azide Effects on Water and Rb(^{36}) Efflux</td>
<td>25</td>
</tr>
<tr>
<td>7.</td>
<td>Azide Effects on Water and Rb(^{36}) Efflux</td>
<td>27</td>
</tr>
<tr>
<td>3.</td>
<td>DNP Effects of Water and Rb(^{36}) Efflux</td>
<td>29</td>
</tr>
<tr>
<td>9.</td>
<td>DNP Effects on Water and Rb(^{36}) Efflux</td>
<td>31</td>
</tr>
</tbody>
</table>
CHAPTER I

INTRODUCTION

For a number of years opinions have been widely divergent concerning the mechanisms of ion and water uptake in plant cells and the relationship between them. A number of workers have supported the theory that both water and ion uptake are passive, i.e. materials enter tissue solely by diffusion due to concentration gradients, or by some pull or force due to the transpiration stream. Opposing this view there are a number of workers who have presented evidence for an active uptake of both substances. Although the actual movement in this process is passive, some workers believe that cells may expend energy in order to arrange the appropriate conditions for influx or efflux to occur. This arrangement may be accomplished in several ways: (1) osmotic potentials may be accumulated at a given internal site, thus establishing osmotic gradients; (2) the electrical charges at a given surface may be altered in a manner in which only certain materials may enter, thus indicating some kind of selection process; (3) physical changes in the protein or lipid structures of membranes; (4) activation of enzyme carrier systems.

In recent years the passive entry theories have received a great deal of support, (14, 17, 18, 32). Hylmo (14) supports the idea that most
water, and consequently, ion uptake occurs due to the pull of the transpiration stream. In contrast to Hylmo's work, Brouwer (5) contends that water and ion uptake are independent of each other. He has also reported water and oxygen uptake to be independent of each other. Thus he considers both water and ion uptake to be independent of metabolic processes. Ordin and Kramer (23) reported inhibition of water uptake in *Vicia faba* roots treated with cyanide and dinitrophenol. However, they attributed the inhibitory effects of these agents to a decrease in water due to a decreased osmotic gradient. Lopushinsky and Kramer (20), using pressure to artificially induce entry of water into tomato roots, found the amount of phosphorus moved inward at a pressure of thirty pounds per square inch to be 2.0 times the amount moved by active transport in the absence of pressure. These workers further stated that the concentration of salts obtained from the exudates of roots under pressure was less than the concentration of the external solution, indicating the existence of a barrier in roots which prevents the free movement of ions into the xylem. This barrier is generally considered to be the endodermis. Crafts and Broyer (5) likewise regarded the endodermis as a barrier to free ionic movement; however, they contended that ions move mainly in the cytoplasm of cortical tissue. In 1956, Arisz (1) proposed the symplast theory in which he contended that ions entered the cytoplasm by an active process and moved passively through the symplast to be finally released into the xylem by an active process. Lundegardh (23) regarded the epidermal
boundaries rather than the endodermis as a barrier to free ion diffusion.

Evidence for the dependence of water uptake and efflux on metabolic processes was demonstrated by Lott and Rosene (22, 29) in studies in which they demonstrated that anoxia inhibited water efflux in isolated onion roots. Further evidence for either the direct or indirect dependence of water uptake on metabolic processes has been supported in studies by Rosene (27, 23) utilizing metabolic inhibitors such as azide and cyanide. In these same studies, she reported that the weaker concentration of the same inhibitor stimulated both water and oxygen uptake. Lott (21) has shown that both water and oxygen uptake may be stimulated by the redox dye, pyocyanin. The parallel stimulation and inhibition of water efflux and oxygen uptake with substances known to alter energy yielding reactions in cells indicates the possibility of a close relationship between oxidative metabolism and water uptake. This idea is further substantiated by the work of Broyer and Hoagland (4) who demonstrated that the transfer of materials to the stele is affected by both temperature and the supply of oxygen in much the same manner as is respiration.

Evidence for the dependence of ion transport mechanisms on oxidative metabolism has likewise been indicated by the numerous reports on effects of substances known to inhibit respiration. A number of workers have shown dinitrophenol to significantly inhibit ion uptake (2, 7, 12, 13, 15, 16). Azide (31) and cyanide (17) have also been shown to inhibit ion uptake.
Further evidence for the expenditure of energy in ion transfer and that a barrier to free movement exists has been provided by experiments in which the concentration of ions in the vascular sap has been shown to exceed the concentration in the outer medium. Russell and Barber (30) reported that the solute concentration of the exudate from excised roots exceeded that in the ambient medium by a factor of between 10 and 15; an osmotic effect was thus clearly demonstrated which could not have occurred if ions were capable of free diffusion. In experiments with excised barley roots Broyer (3) showed that the concentration of bromide in the exudate amounted to thirty times that found in the external solution.

Epstein's work (9) showing the interference of potassium with rubidium is further evidence for active uptake. He showed that rubidium rates varies in response to the rubidium concentration; a linear relationship was noted when a double reciprocal plot of absorption versus substrate (rubidium) concentration was made, thus indicating a possible competition for an identical carrier site.

In addition to the initial rapid (passive) and the slow metabolic (active) components of absorption, recent workers (6, 9, 14) have identified a third component which appears to involve a rapid diffusion or Donnan exchange through the cell wall into a so-called free space area in the cell wall. Priestley (26) as early as 1920 proposed a theory in which he postulated that ions pass freely into and through the cell walls of the cortex to
the endodermis after which the ions are transported through the endodermal protoplasts into the stele. While there is considerable support for the concept of free space, there is considerable disagreement among the free space proponents as to its extent and location. Free space has been estimated to account for from 5 to 30 percent of total tissue volume (6, 10, 14, 19). Lundegardh (24) believes it is located in the cytoplasm external to the tonoplast while Levitt (19) contends that it is located in the cell wall. Hylmo (14), Epstein (10), and Kramer (17) support the theory of Hope and Stevens which states that it is not the cell wall but the cytoplasm into which most of the ions diffuse.

The purpose of this study was to learn more about the possible relationships between water and ion uptake in plants and to demonstrate whether water uptake and ion absorption are actually distinct and independent processes. The popular belief that water and ion uptake are independent processes probably resulted from the fact that most investigations have been concerned solely with ion uptake. Moreover, most of the work dealing with ion accumulation or uptake has been done by soaking discs of tissue such as potato or beet and determining ion concentration changes in the external solution or by chemical analysis of ashed tissues. Thus there are relatively few publications dealing simultaneously with water and ion uptake. It was felt that a micro-potometric method would be considerably more suitable to accurately measure both water and ion movements in isolated root systems than the methods used by the foregoing
investigations. It should be mentioned that the advantage in studying excised roots rather than intact roots would be to negate any possible transpirational forces by the shoot.

In brief, the aims of this study were: (1) to determine simultaneously by means of a micro-ootometric method the rates of water and various isotopic ion effluxes from isolated onion roots; (2) to determine and compare the effects of metabolic inhibitors on both processes simultaneously; (3) to ascertain the similarity or dissimilarity of mechanisms involved with these two processes; (4) to ascertain the role of active metabolism in each of these processes; (5) to shed light on possible gearing mechanism(s) between metabolic and transport processes.
CHAPTER BIBLIOGRAPHY


CHAPTER II

METHODS AND PROCEDURES

General Methods

Onion bulbs (Allium cepa, var., White Ebenezer) were sprouted in continuously aerated, full strength Hoagland's solution (2). Bulbs weighing between four and six grams were supported on perforated plexiglass strips over an aquarium containing twenty liters of the nutrient solution. The bulbs were so positioned on the strips that the stem bases were slightly immersed. The growth chamber was darkened in order to obtain maximum root growth and minimum leaf growth. Temperature during growth was 23 ° ± 1 ° C with the relative humidity ranging between 55 percent and 60 percent. At the time of the experimentation, the roots were usually five days old. Only healthy, stout roots ranging from seventy-five to one hundred millimeters in length were used in the experiments. The chamber was maintained in a constantly lighted room. The source of light was a battery of cool, daylight fluorescent lamps.

Inhibitor Solutions

Sodium azide and 2, 4-Dinitrophenol (DNP) obtained from Eastman Organic Chemical Company of Rochester, New York, were used as
metabolic inhibitors in these studies. A series of at least ten experiments were run with both inhibitors at concentrations of $1 \times 10^{-3} \text{ M}$, $1 \times 10^{-2} \text{ M}$, $5 \times 10^{-4} \text{ M}$, and $5 \times 10^{-5} \text{ M}$ at a pH of 6.0. In order to put DNP into solution it was necessary to adjust the pH to at least 10.0 with dilute base, after which the pH was readjusted to 6.0 with dilute acid. With solutions of Rb$^{36}$, pH adjustments were made with dilute KOH, and the Na$^{22}$ inhibitor solutions were adjusted with dilute NaOH in order to eliminate any possible competition of Na$^+$ with Rb$^+$ for entrance into the root or vice versa. It was stated above that the inhibitor solutions were adjusted to a pH of 6.0. This pH was used in order that as many molecules as possible be present in the undissociated and therefore more penetrable state. Beevers (1) has shown that the undissociated form of molecules seem to be the most active in entering tissue. Simon and Beevers (6) state, moreover, that inhibitors are more effective if they are employed at a pH which is lower than their pK. It was not experimentally feasible in the present study to reduce the pH below the pK of the inhibitors used, but the ideal condition was approached as near as the required physiological condition would allow. Only fresh, aerated test solutions were used in the experiments.

Different concentrations of both sodium azide and DNP were used since, according to Hackett (4), the concentration is important in determining the nature of the response. Beevers (1) further stated that the response to an inhibitor depends on the amount actually entering a cell,
and that this is altered by changing either the concentration of the inhibitor or the pH outside the cell.

Radioisotope Solutions

The radioactive rubidium was obtained from the Oak Ridge National Laboratory of Oak Ridge, Tennessee, as Rb\textsuperscript{36}Cl in HCl solution. The Stock Rb\textsuperscript{36}Cl concentration was 15.20 mc/ml with a specific activity of 2093 mc/g. The stock solution was diluted to a concentration of 3 uc/ml and adjusted to pH 6.0 with 10 percent KOH. The radioactive sodium was obtained from the Nuclear Science Engineering Corporation of Pittsburg, Pennsylvania, at a concentration of 1.01 mc/ml and with a specific activity of 1.8 mc/mg. The stock solution was diluted to a concentration of 2 uc/ml and the pH adjusted to 6.0 with 10 percent NaOH.

Samples of exudate containing the radioactive materials were placed on siliconized one inch planchets and rapidly dried under infra-red heat in order to insure good sample geometry. The planchets were then placed in a one and one-half inch shielded counting chamber and analysed for activity with a Radiation Equipment and Accessories Corporation transistorized Model E115 Scaler. The Geiger-Mueller tube used was an end window type (1.4 mg/cm\textsuperscript{2}). The tube was calibrated every six weeks in order to determine the proper operating voltage plateau. With each sample a total of at least 1,000 counts was made in order to give reliable count reproducibility. Corrections for radioactive decay of Rb\textsuperscript{36} were
necessary due to its relatively short half-life of 13.6 days. These corrections were made with the aid of a semi-logarithmic plot in which the relative activity (per cent) was plotted on a logarithmic scale against the time on a linear scale. The per cent activity on any day could then be directly determined from the graph.

Experimental Procedure

Prior to each experiment the roots were excised under water and placed in a pyrex dish containing aerated distilled water for one hour. This was done to permit each root to recover from the excision and to reduce possible water deficits. After this equilibration, an individual root was placed into a micro-potometer (Figure 1-A) similar to that described by Rosene (3). The entire process of loading the potometer was carried out as rapidly as possible to prevent dehydration and, thereby, a water deficit in the root. In most experiments it took approximately thirty to forty-five seconds to load. The total length of the root immersed in each experiment was 40 millimeters. Immediately after loading the potometer was immersed in a water bath within a closed plexiglass chamber as seen in Figure 1-B. The potometer was supported in the chamber in such a manner that three quarters of the potometer was immersed in the water bath; thus it was assumed that the temperature of the well solution was equal to that of the water bath ($23^\circ + 1^\circ$ C). After positioning in the chamber, the loaded potometer was allowed to equilibrate for two hours before the experimental
Fig. 1-A--Micro-potometric Apparatus

Fig. 1-B--Micro-potometer in Humidity Chamber
period was initiated. The construction of the chamber was such that solutions in the well surrounding the root could be changed without removing the potometer from the water bath and moist chamber. The rubber stoppered port on the right side of the chamber as seen in Figure 1-B allows this fluid exchange.

The volume of water efflux was measured at ten minute intervals by means of a horizontal microscope equipped with a calibrated micrometer eyepiece. Since the diameter of the potometer could be determined, a constant was derived ($r^2$). Thus the volume of water efflux (exudate) pumped for any unit of time could be determined by multiplying the constant by the distance the meniscus moved in the potometer. Exudate samples for the determination of the various ion concentrations were withdrawn from the potometer with a micro-syringe. These samples were then either analysed flame photometrically or their radioactivity determined with a Geiger-Mueller tube and scaler.

Initially, a series of experiments was run in order to determine the rates of water efflux under a given set of laboratory conditions. The rates of water efflux obtained were then compared with those reported by other workers. Aerated distilled water was used in the potometer well in this series, and the pH was adjusted with dilute HCl to a pH of 6.0. The duration of each experiment was four hours.

A second series of experiments was run in order to establish the rates of non-isotopic potassium and sodium efflux from the roots. In each
of these experiments, a one hour control period with distilled water in
the well was followed by the addition of KCl or NaCl, each at a concent-
tration of 5 meq/liter and pH ranging between 6.0 and 6.5. At thirty or
sixty minute intervals, depending on the individual experiment, one micro-
liter samples of exudate were taken from the potometer with the aid of a
Hamilton #7001 microsyringe. The one microliter aliquotes were then
diluted in 2 ml. of de-ionized water and analysed on a Coleman Flame
Photometer (Model 21) for K⁺ or Na⁺. The concentrations accordingly
were recorded in terms of milliequivalents of ion per microliter sample
(meg/ul).

A third series of experiments involved a study of the efflux of the
two isotopic ions from isolated roots. In the first group of experiments,
\(^{36}\)Rb was used as an isotopic tracer for potassium. Validity for the use
of Rb is suggested by the work of Higinbotham (3) who conducted experi-
ments on the absorption of \(\text{Rb}^+\) and \(\text{K}^+\) from solutions of \(\text{RbCl}, \text{KCl},\) and
mixtures of these using both \(\text{Rb}^{36}\) and \(\text{K}^{42}\) tracers. The use of \(\text{Rb}^{36}\) as
a \(\text{K}^+\) analogue has also been confirmed by the studies of other workers.

Radioactive \(\text{Na}^{22}\) was used in the majority of the Na experiments.

A fourth series of experiments involved a study of the effects of
two metabolic inhibitors, sodium azide and dinitrophenol, on water and
ion efflux from isolated roots. Each period lasted sixty minutes except
in a few experiments in which the test period duration was two hours. The
inhibitor solutions were introduced and withdrawn without removing the
potometer from the water bath and moist chamber. This was accomplished with small rubber suction bulbs through small rubber stoppered portals located in the right side of the humidity chamber as seen in Figure 1-B. The time required for this operation ranged between thirty and forty-five seconds.

In most of the experiments involving the effects of metabolic inhibitors, water efflux was recorded at ten minute intervals while 1 ul isotope samples were removed every sixty minutes since sufficient time had to be allowed to permit enough exudate to fill the potometer for a sample to be taken. In another set of experiments, water and ion efflux were both determined and ion samples removed every hour. Whenever necessary, following the removal of a microliter sample from the potometer, another microliter of deionized water was added to the potometer in order to replace the lost volume of exudate.
CHAPTER BIBLIOGRAPHY


CHAPTER III

RESULTS

Over 175 roots were used in this study. Prior to the actual test experiments, ninety roots were used to establish control conditions and to overcome technical difficulties. Exudation rates for both water and cations under control conditions also had to be established. Technical problems such as loading a root in a potometer without injury to it and in a minimal amount of time in order to prevent water deficits had to be mastered. Prevention of bubble formation in the potometer was a problem to be considered occasionally. Only roots which demonstrated a constant rate of efflux during the control period were used for test experiments.

The data obtained in these studies were presented in three basic ways: (1) time course curves presenting typical efflux rates for both water and ions; (2) bar graphs depicting mean values for one hour exudations of both water and ions; (3) a summary table depicting mean values for rates of efflux and the degree of inhibition or enhancement in water and ion efflux following inhibitor application. Water efflux rates are presented graphically as uls/min for 40 mm submerged root lengths and as uls/hour/mm root length. All radioactive ion efflux activities are presented as cpm/ul of sample while flame photometric ion determinations are presented as meq/ul.
General Control Experiments

The curve in Fig. 2 depicts the mean water efflux values for twenty roots. The solution surrounding the roots in the potometer well was triple de-ionized water. Efflux readings in each experiment started after a two hour equilibration period. In a series of determinations using five roots, 5 meq/l KNO$_3$ in the ambient solution did not appear to produce any significant effect on the rate of exudation. This was done in order to determine any possible effects of K on the exudation of water.

A time course curve for K ion efflux expressed as meq/ul of sample as determined with a flame photometer is presented in Fig. 3. Each value represents the mean values of sixteen roots. An ambient solution of KNO$_3$ at a concentration of 5 meq/l was used in each experiment. The concentrations of K$^+$ pumped with different roots, the values for any single root appeared to be relatively constant for the duration of the four hour experiments. Differences were probably due to the inherent characteristics of different roots.

Attempts to determine flame photometrically the amount of Na ion in the exudate of roots placed in 5 meq/l NaCl were unsuccessful. The roots apparently took up so little Na$^+$ that the concentration in the exudate was too dilute to be detected with the flame photometer. Studies utilizing Na$^{22}$Cl at a specific activity of 2 uc/ml yielded similar results; however, low levels of Na$^{22}$ activity could be detected in exudate samples. The count rates for individual experiments ranged between nine and
Fig. 3—Potassium Efflux From Isolated Onion Roots

[min 120 180 240

[10 20 60

meg / yr (mean)
twenty-four counts per minute. The rate for four hour periods remained relatively constant.

Water and Na\textsuperscript{22} Efflux

Figure 4 presents typical time course curves depicting the effects of 1 \( \times 10^{-2} \) sodium azide on isolated roots. A total of nine experiments were run in this series. Water efflux is noticeably increased above the control values, whereas no significant change in Na\textsuperscript{22} efflux is evident. The water efflux during the recovery period appeared to be slightly above the control values.

Figure 5 shows typical effects of 1 \( \times 10^{-4} \) M DNP on water and Na\textsuperscript{22} efflux. Water efflux is significantly inhibited by DNP while Na\textsuperscript{22} efflux remains relatively unchanged. Water efflux appeared to recover immediately upon replacing the test solution with the control solution.

Water and Rb\textsuperscript{86} Efflux

Time course curves showing typical results obtained with different concentrations of sodium azide are presented in Fig. 6. A total of 37 roots were tested in this series. It is evident that at all four concentrations azide brought about either an enhancement or an inhibition of water efflux almost immediately. This observation indicates a relatively rapid inhibitor penetration into the root. At both concentrations of azide which stimulated water efflux, 5 \( \times 10^{-5} \) M and 1 \( \times 10^{-4} \) M, the maximum effect was observed approximately forty minutes after application of the test
Fig. 4—Azide Effects on Water and Na\textsuperscript{22} Efflux

Fig. 5—DNP Effects on Water and Na\textsuperscript{22} Efflux
Fig. 6--Azide Effects on Water and Rb$^{86}$ Efflux
solution. Subsequently, a gradual decline in the stimulatory effect was noted. During the recovery period, control rates were regained and maintained for periods of up to one hour. Azide at $5 \times 10^{-4}$ and $1 \times 10^{-3}$ M concentrations brought about an immediate and sustained decrease in the rates of water efflux. Complete recovery was never observed in any experiment although the overshoot phenomenon was noted in some roots. Both Rb$^{36}$ and water efflux were enhanced and inhibited at similar concentrations; however, the magnitude of inhibition in regard to ion efflux was less. Although water efflux failed to recover to even one half of the control values, Rb$^{36}$ efflux recovered to levels slightly above that of the controls in all experiments.

Figure 7 shows the effects of the different concentrations of sodium azide on both water and Rb$^{36}$ efflux. The values presented in this graph are mean values, representing the results of 37 experiments in which both water and ion effluxes were determined after one hour each of control, test, and recovery periods. The ambient solution concentrations of Rb$^{36}$ in each three phases of the experiment was the same. It is evident from these data that the different concentrations of azide have vastly different effects on both water and Rb$^{36}$ efflux values. A $5 \times 10^{-5}$ M concentration of azide produced a marked increase in the mean amounts of both water and Rb$^{36}$. The extent of increase with both water and Rb$^{36}$ appeared to be approximately the same; however, the extent of recovery of Rb$^{36}$ efflux appears to be somewhat lower than that of water efflux. The general
Fig. 7--Azide Effects on Water and Rb\textsuperscript{86} Efflux
shapes of the graphs obtained with azide at $1 \times 10^{-4}$ M are similar to those at $5 \times 10^{-4}$ M. However, the extent of increase of both the test and recovery periods are not as great as with $5 \times 10^{-5}$ M. At this concentration the extent of $\text{Rb}^{36}$ recovery was only slightly greater than the control. At a concentration of $5 \times 10^{-4}$ M water and $\text{Rb}^{36}$ were noticeably inhibited and showed almost complete recovery.

An increase inhibition of water and $\text{Rb}^{36}$ efflux was noted at $1 \times 10^{-3}$ azide.

Figure 8 presents typical time course curves showing the effects of various concentrations of 2,4-dinitrophenol (DNP). As with the azide solutions, all concentrations of DNP brought about an alteration in both the water efflux and $\text{Rb}^{36}$ efflux. Noticeable effects were observed within ten minutes after the inhibitor treatment which again suggests rapid tissue penetration by DNP. DNP at $5 \times 10^{-5}$ M increased water efflux slightly above control values. The recovery period showed an increase in water efflux that reached a maximum value at twenty minutes followed by a gradual decline approximating control values. The same concentration of DNP brought about a slight increase in $\text{Rb}^{36}$ during both the test and recovery periods. At concentrations of $1 \times 10^{-3}$ M, $5 \times 10^{-4}$ M, and $1 \times 10^{-5}$ M, DNP brought about a profound and immediate inhibition of water efflux. Recovery was relatively immediate and complete with slight overshooting in some instances. $\text{Rb}^{36}$ efflux appeared to increase in both the test and recovery periods following application of $5 \times 10^{-5}$.
$1 \times 10^{-4}$, and $5 \times 10^{-4}$ M DNP. The greatest depression in both water and Rb$^{36}$ efflux was seen in experiments involving roots treated with $1 \times 10^{-3}$ M DNP. Water recovery was relatively slow, reaching near control levels after thirty minutes. Rb$^{36}$ efflux also showed recovery within one hour.

Figure 9 shows bar graphs presenting efflux rates of water and Rb$^{36}$ in roots treated with different concentrations of DNP. Each bar represents mean values for at least six roots. It is clear that at the lowest concentration ($5 \times 10^{-5}$ M), DNP brought about a slight increase in both water and Rb$^{36}$ efflux. As the concentration of DNP was increased, there was an enhanced depression of water efflux rates. It is interesting to note, however, that only at the highest concentration of DNP ($1 \times 10^{-3}$ M) was there noted a statistically significant depression in the rate of Rb$^{36}$ efflux. In general, all of the roots demonstrated full recovery in both water and Rb$^{36}$ efflux; some instances of overshooting occurred in both processes. Recovery occurred more quickly following application of the weaker concentrations of the inhibitor.

The results showing the effects of both inhibitors on water and Rb$^{36}$ efflux are summarized in Table I. The data are presented in terms of mean percentage of increase or decrease in the rates of efflux for both water and Rb$^{36}$ following application of the inhibitors. Each figure listed denotes mean values for at least seven roots.
Fig. 9--DNP Effects on Water and Rb$^{86}$ Efflux
Several important findings are apparent in this table. Dinitrophenol at each different inhibitor concentration appears to be a more effective inhibitor of water efflux than sodium azide. This finding is less apparent in regard to Rb$^{86}$ efflux where it is evident that only at a relatively high concentration (1 x $10^{-3}$ M) does significant depression in ion efflux occur. Another significant observation is the fact that at similar concentrations, the water transport is more easily effected by the inhibitors than the Rb$^{86}$ transport. The strongest concentration of azide (1 x $10^{-3}$ M) reduced water efflux 61 per cent and Rb$^{86}$ efflux 41 per cent whereas at the same molarity, DNP reduced water efflux by 79 per cent and Rb$^{86}$ efflux only 32 per cent. The differential in effect in both processes at the same concentration is slightly higher, about 50 per cent, for dinitrophenol.
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<th>Mean Rb$^{86}$ Efflux</th>
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<td>.025 ± 0.004</td>
<td>.010 ± 0.002</td>
<td>- 54</td>
</tr>
<tr>
<td>5 X 10^{-4} M</td>
<td>10</td>
<td>.031 ± 0.005</td>
<td>.031 ± 0.003</td>
<td>- 50</td>
</tr>
<tr>
<td>1 X 10^{-3} M</td>
<td>3</td>
<td>.032 ± 0.004</td>
<td>.007 ± 0.001</td>
<td>- 79</td>
</tr>
</tbody>
</table>

* Control Rate - Test Rate \times 100
** Standard Deviation
*** Differences between control and test are statistically significant
CHAPTER IV

DISCUSSION

The rates of water efflux during the control periods of all the various noted in efflux rates for individual roots were probably due to individual differences in the roots themselves since each root pumped at a relatively constant rate, some for periods of four hours or longer. These variations have been reported by other workers (19, 23). The efflux rates reported here closely approximate those reported by Lott (19) in studies using 45 mm submerged portions of onion roots. Rosene (23), using 55 mm submerged portions of the same tissue, obtained slightly higher efflux rates. The fact that these experiments were performed in a lighted room could account for lower efflux rates since according to Brouwer (1), light will inhibit water efflux. However, the close approximation of these results with those of other workers, who performed the experiments in the dark, tends to indicate that light may not be an important factor.

The literature is abundant with publications concerning K+ absorption or accumulation in plant tissue. However, it is difficult to make accurate comparisons with these studies since they are
concerned with absorption in tissue slice instead of the amount present in the exudate of excised roots. In a recent study by Jackson and Adams (16), barley roots were reported to absorb approximately 0.525 meq/1-gm-hr. This closely approximates the values of 0.60 to 0.80 meq/1-gm-hr found in this study. Higinbotham, Latimer and Eppley (13) reported approximately 5.5 ueq/gm-hr in studies with pea epicotyls.

In contrast to the copious amounts of work concerning potassium absorption, very few publications deal specifically with Na absorption or accumulation. The low values for Na exudation observed in this study might be due to the relatively small amount of tissue used. Using significantly greater amounts of tissue, Jacobson et al. (17) reported Allium cepa to absorb Na to an extent of 11.4 meq/Kg during four hour experiments. This value would approximate 0.0023 meq/g/hr which is below the limits of sensitivity of the detection equipment used in this study. Jackson and Adams (16) reported Na+ absorption in studies with barley roots, but they also used larger amounts of tissue (3 to 4 grams). The failure to demonstrate Na+ in exudates is not too unusual in that Na+ is known not to be generally required for plant life (8). The absence of K+ from solutions used in the Na+ uptake studies might have accounted for the low efflux of Na+ uptake studies might have
accounted for the low efflux of \( \text{Na}^+ \). An explanation for this phenomenon has been proposed by Epstein (8). He stated that:

While potassium and rubidium invariably compete with each other in the process of absorption, sodium at low concentrations of the ions does not readily compete with these two. This means, according to the earlier hypothesis, that the binding sites for potassium and rubidium are identical, but sodium is bound by different sites. However, at higher concentrations of rubidium or potassium sodium competes, and this led to the conclusion that even in the absorption of a single ion species, say potassium or rubidium, two types of sites are involved. One, with a high affinity for potassium (or rubidium), is responsible for most of the absorption at low concentrations, and has little affinity for sodium ions. At higher concentrations another site assumes increasing importance, and this site can bind sodium as well—hence then competition by sodium at the higher concentrations of potassium or rubidium.

MacRobbie and Dainty (20) studied the transport of sodium, potassium, and chloride in the cell of the giant alga, \textit{Nitella obtusa}. Using electrochemical potential measurements, they reported potassium to be distributed "approximately as expected," but reported sodium to be actively transported outwards and chloride actively transported inward. The exclusion of sodium from plant tissue poses a puzzle regardless of the theory one accepts for its absence.

The effects of azide and DNP on water efflux shown in this study were similar in effect although different in magnitude; i.e. acceleration of water efflux occurred in dilute concentrations
of both inhibitors while stronger concentrations brought about inhibitions which were proportional to concentrations of each inhibitor. Rosene (13) showed effects with azide similar to those obtained in this study. She reported maximum inhibitions of water efflux with 0.05 M azide. She also showed recovery at this concentration; whereas, in this study, recovery at $1 \times 10^{-3}$ M was delayed and very slight. This was thought at first to be due to experimental error, but the consistency of this observation in all eight experiments performed at this concentration tend to confirm these findings. Her results with $1 \times 10^{-4}$ M azide treated onion roots are in close agreement with those in the present study.

The inhibition of water uptake by DNP as observed in this study was not consistent with the findings of Brouwer (2) who reported that concentrations of DNP at $1 \times 10^{-2}$M and $1 \times 10^{-3}$M had no influence on water uptake, even after eight hours. Ordin and Kramer (22) attribute the depression of water movement in DNP treated Vicia Faba roots to a depression of salt secretion rather than on water movement per se. In regard to the effects of metabolic inhibitors on the water permeability of roots, various workers (2, 10) have found that respiratory inhibitors, in general, may reduce the water permeability of photoplasma.
The effects of DNP on ion absorption have been discussed by numerous workers (4, 11, 12, 14, 16). Jackson and Adams (16) reported a 90 per cent decrease in K (Rb) absorption in barley roots treated with $1 \times 10^{-4}$ M DNP. In this study, however, Rb absorption was increased at this concentration. The difference in effect is most likely due to pH differences. Jackson and Adams maintained the pH at 5.0 while it was 6.0 in this study. Norris (21), in respiratory studies with onion root tips, has shown pH to be extremely critical in determining the action of an inhibitor. For instance, he found respiration slightly stimulated with $1 \times 10^{-4}$ M DNP at a pH of 6.0 in contrast to considerable inhibition at pH 4.5. Simon and Beevers (24) state that at any single pH, stimulatory and inhibitory concentrations of DNP can be found; likewise, a single concentration of DNP may produce either stimulation or inhibition as the pH is varied. The nature of DNP action is thus based on the ability of it to penetrate the cell which (in turn) is dependent on its ionized state.

Canning and Kramer (4) reported 90 per cent inhibition of phosphorus - 32 accumulation in sweet corn roots with $1 \times 10^{-4}$ M DNP. The pH for these determinations was not given. Higinbotham (11), working with etiolated pea epicotyls, demonstrated $1 \times 10^{-5}$ M DNP at pH 4.8 to decrease Rb+ absorption approximately 70 per
cent. This inhibition was undoubtedly due to the low pH used. At the same concentration of DNP Higinbotham demonstrated oxygen consumption to be enhanced. He considered this as being indicative of a possible relation of ion uptake to oxidative phosphorylation or dephosphorylation. He further stated that the lowest levels of DNP which enhanced respiration also depressed rubidium uptake, indicating that the action of ATP may be to induce hydrolysis of ATP in a non-useful reaction rather than to block ATP formation. This theory that DNP induces the hydrolysis of ATP has recently been refuted by studies of DNP on oxidative phosphorylation by Eisenhardt and Rosenthal (7). In this study they showed the initial rapid phase of phosphorylation, the so-called "adenosine triphosphate jump," to be undisturbed when DNP was added to rat mitochondria just before addition of ATP. However, the steady-state phosphorylation was abolished, thus leading them to the conclusion that uncoupling agents operate by interfering with the synthesis of high-energy intermediates, not by hydrolyzing or otherwise inactivating them.

The suggestion that there is no specificity for uncoupling phosphorylation within the respiratory chain in the several stages of electron transport between DPNH and O₂ (5), makes it difficult to localize the action of DNP. However, it is known that DNP does not interfere with the phosphorylations associated with the substrate level oxidations of phosphoglyceraldehyde, pyruvate, and
-ketoglutarate (9). Thus it is possible that one of these Kreb cycle or glycolytic intermediates may in some way be associated with K+ uptake, since K+ uptake has been demonstrated in this study to be resistant to DNP at concentrations which are known to inhibit other phosphorylative reactions (9). The Rb+ inhibition at 1 x 10^-3 M may be explained by the fact that DNP at high concentrations has been shown to inhibit respiration due to the hydrolysis of essential nucleotides and coenzymes (18). Thus it is possible that ion uptake may be in some way related to terminal respiration rather than directly on phosphorylative reactions.

The above hypothesis is further substantiated by the azide inhibition of Rb$^{36}$ efflux. Azide is an inhibitor thought to inhibit terminal cytochrome oxidases. However, azide does affect other loci, as shown by its ability to alter the rate of fermentation and in some instances to uncouple oxidative phosphorylation (9).

In regard to the lack of correlation and the extent of dependence of ion uptake on water uptake, Broyer and Hoagland (3) proposed that plants initially low in salt content exhibit ion absorption independent of water uptake. They further demonstrated that plants whose initial salt content was relatively high showed increased ion absorption with increased water uptake. Hylmo's (15) theory that salt enters plant tissue slowly by mass flow due to the transpiration stream fails to explain completely the active pumping of xylem sap reported by
Crafts and Broyer (6) as well as the present data in which excised roots were used. Brouwer (2) proposed that salt uptake is inhibited by lower concentrations of inhibitor than is water uptake, and he believes that this indicates that the inhibition of water uptake is an indirect process which is not directly dependent on respiratory mechanisms. In certain experiments in this study utilizing 1 X 10^-3 M sodium azide, salt efflux was demonstrated in the absence of any measurable water efflux. Furthermore, long term experiments (up to five hours) conducted with only aerated triple deionized water in the potometer well precluded the dependence of water uptake on osmotic gradients set up by salts. Extended periods of efflux in aerated de-ionized water has also been demonstrated by Lott (20).

The inhibition of both Rb\(^{36}\) and water efflux by azide suggests that both processes are in some way related to terminal respiration. On the other hand, the similarity of water uptake by both azide and DNP indicates that reactions other than those concerned with phosphorylation and terminal respiration may be involved. It seems clear, therefore, that net water efflux is not directly related to Rb absorption. The DNP inhibition of water at concentrations which stimulate ion efflux also indicates separate mechanisms, or at least the lack of direct dependence of one process on the other. However, the fact that the rates or net efflux of water and ions may differ is
not conclusive evidence that there is no relation between the two processes. The possibility of separate metabolic mechanisms for water and ion efflux is indicated in this study.

Summarily these findings indicate that:

(1) One can alter the process of water and ion transport in isolated root systems by application of metabolic inhibitors;

(2) The alterations brought about by inhibitors may be inhibitory or stimulatory depending on the concentrations used;

(3) Water transport is more sensitive to sodium azide and DNP than ion transport, indicating separate mechanisms controlling the two processes; and

(4) Active transport, at least in isolated root systems, is clearly indicated by the data presented.
CHAPTER BIBLIOGRAPHY


CHAPTER V

SUMMARY

1. A micro-potometric method is described which was used to determine simultaneously the rates of water and isotope efflux from isolated onion roots (Allium cepa). The isotopes used were Na$^{22}$ and Rb$^{86}$. The latter isotope was used as a potassium analogue.

2. The effects of two metabolic inhibitors, sodium azide and dinitrophenol, on the two processes were studied.

3. Inhibitor solutions at the following concentrations were tested: $1 \times 10^{-3}$ M; $5 \times 10^{-4}$ M; $1 \times 10^{-4}$ M; and $5 \times 10^{-5}$ M. All test solutions were fixed at pH 6.0.

4. It was found that dilute concentrations of both inhibitors brought about an enhancement in water and isotope efflux. Both processes were significantly inhibited by the strongest solutions. Recovery was noted in all experiments.

5. Water efflux appeared to be more sensitive to both inhibitors than isotope efflux.

6. The results were discussed in terms of different operating mechanisms involved with the two processes as indicated by the
differential in the magnitude of effect brought about by the inhibitors.

7. The data clearly indicate active transport of water and ions in isolated root systems, involving an expenditure of energy on the part of the absorbing cells.
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