# RESPIRATION STUDIES OF NORMAL AND PHOSPHATE DEFICIENT ISOLATED LEAF CELLS

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# RESPIRATION STUDIES OF NORMAL AND PHOSPHATE DEFICIENT ISOLATED LEAF CELLS

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A Thesis

Presented to

The Graduate Division

Drake University

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In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

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by

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August 1966

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# CHAPTER I

## INTRODUCTION

For many years, scientists have known that plant foliage will absorb solutes applied to their surfaces. This important function has always been greatly overshadowed by studies on the primary function of plant foliage, photosynthesis.[1] In recent years, however, there has been much interest in the study of the pathways and mechanisms by which solutes applied to foliage are absorbed.

Jyung and Wittwer, in 1963, demonstrated that foliar absorption was an active uptake process.[2] Using radioactive isotopes and a newly developed leaf-washing and leaf-immersion technique, they found that the over-all absorption action is directly related to cell metabolism, and that proteinaceous carriers probably play an important role in the uptake process.

The studies of Jyung and Wittwer did not show a differentiation between absorption due to the active leaf cells and absorption due to stomatal activity, cuticular

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[1] W. H. Jyung and S. H. Wittwer, "Pathways and Mechanisms for Foliar Absorption of Mineral Nutrients," Agricultural Science Review (Second Quarter, 1965), 25-36.

[2] W. H. Jyung and S. H. Wittwer, "Foliar Absorption - An active Uptake Process," American Journal of Botany, LI (April, 1964), 437-444.

diffusion, cracks in the leaf surface or translocation in the leaf. Then, in 1965, Jyung, Wittwer and Bukovac, using an isolation technique modified after Zaitlin, isolated individual cells from plant leaves. This provided a means for studying leaf absorption without the influence of other surface factors. Their work supported the previously proposed theory that absorption by plant foliage is an active uptake process coupled with cell metabolism.[1]

The problem arises as to how this active absorption process is coupled with the metabolic processes. This investigation of plant leaf cells was concerned with respiration, which is only one of the many phases of the metabolic processes, and with the phosphate ion, which is only one of the many ions absorbed by the leaf cells. It was the purpose of this investigation to study the relationship between the active uptake process of the phosphate ion into the leaf cells and the respiratory activity of these cells.

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[1]W. H. Jyung, S. H. Wittwer, and M. J. Bukovac, "Ion Uptake by Cells Enzymically Isolated from Green Tobacco Leaves," Plant Psysiology, XXXX (May, 1965), 410-414.

## CHAPTER II

## HISTORY

It has been well established that a plant leaf can absorb solutes applied to its surface. Very little is known yet as to the mechanisms by which these solutes are absorbed into the leaf cells.

Most of the work done in this area has been with intact leaves, whole excised leaves, parts of leaves or leaves propagated in tissue culture. Very little work has been done with leaf cells isolated from the surrounding plant tissues.

Chayen, in 1952, devised a technique for isolating plant cells. Using an aqueous solution containing a ten per cent concentration of pectinase and a one per cent concentration of peptone, he was able to successfully separate and isolate single root cells from the surrounding vascular and epidermal tissue.[1] The enzyme, pectinase, separated the cells by hydrolyzing the pectic materials in the cement of the middle lamella which holds plant cells together.

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[1] J. Chayen, "Pectinase Technique for Isolating Plant Cells," Nature, CLXX (December 20, 1952), 1070-72.

That same year, Wood, Gold and Rawlins[1] using these same pectic type enzymes were able to isolate cells from leaves, stems, roots and storage tissue for structural analysis of the cell wall under the electron microscope.

Letham, in 1958, developed a new technique for isolating plant cells by maceration. He found that the chelating agent sodium ethylene diamine tetra acetic acid would dissolve the pectic materials of the middle lamella. Using this material and with light maceration and shaking, he could separate and isolate cells without destroying them or causing changes in the cell volume.[2]

In 1959, Zaitlin applied Chayen's pectinase technique to the separation of leaf cells. With the pectinase solution, he successfully isolated and studied tobacco leaf cells. He noted that chloroplasts could easily be seen and that Brownian movement occurred in these cells. He also showed that these isolated cells were capable of supporting colonies of tobacco mosiac virus.[3]

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[1]R. K. S. Wood, A. H. Gold, and T. E. Rawlins, "Electron Microscopy of Primary Cell Walls Treated with Pectic Enzymes," American Journal of Botany, XXXIX (February, 1952), 132-133.

[2]D. S. Letham, "Maceration of Plant Tissue with Ethylene Diamine Tetra Acetic Acid," Nature, CLXXXI (January 11, 1958), 135.

[3]M. Zaitlin, "Isolation of Tobacco Leaf Cells Capable of Supporting Virus Multiplication," Nature, CLXXXIV (April 4, 1959), 1002-1003.

Jyung, Wittwer and Bukovac, in 1965, revised and modified the pectinase procedure for separation of leaf cells of tobacco and bean plants. Using combined techniques of their own, along with techniques and ideas from Zaitlin,[1] Letham,[2] and Burg,[3] they devised a better and more efficient leaf cell isolation procedure. They studied absorption characteristics of these isolated cells. With ions of rubidium and phosphate, these men demonstrated that ion uptake at the cellular level was an active process.[4] This supported their previously proposed theory of the active absorption of ions by the leaf as a whole.[5] They also investigated the action of the antibiotic Chloramphenicol on protein synthesis in isolated plant leaf cells.[6] They found that rubidium ion uptake was reduced and glycine incorporation into protein was inhibited. The primary effect of this antibiotic was on protein synthesis and

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[1]Zaitlin, loc. cit. [2]Letham, loc. cit.

[3]B. P. Burg, E. A. Burg, and R. Marks, "Relationship of Solute Leakage to Solution Tonicity in Fruits and Other Plant Tissues," Plant Physiology, XXXIX (March, 1964), 194.

[4]Jyung, Wittwer, and Bukovac, loc. cit.

[5]W. H. Jyung and S. H. Wittwer, "Foliar Absorption-An Active Uptake Process," American Journal of Botany, LI (April, 1964), 437-444.

[6]W. H. Jyung, S. H. Wittwer, and M. J. Bukovac, "Ion Uptake and Protein Synthesis in Enzmically Isolated Plant Cells," Nature, CCV (February 27, 1965), 921-922.

not on amino acid uptake. This suggested that the absorption of the rubidium ion is closely correlated with protein synthesis.

Kannan and Wittwer, in 1965, studied the effects of urea and chelating agents on iron absorption by isolated leaf cells. They found that urea greatly enhanced iron ion uptake while the chelating agent ethelene diamine tetra acetic acid suppressed its uptake, even though it was not significantly absorbed by the cells.[1]

Also in 1965, Jyung, Dilley, Wittwer and Bukovac studied the effects of certain growth retardants on isolated leaf cells. They found that rubidium ion uptake was not affected, but that oxygen uptake was reduced by these retardants. Certain retardants decreased chlorophyl degradation while others increased it. Certain retardants were found to greatly increase the respiratory activity of the cells. This led to the suggestion that the mode of action of growth retardants at the cellular level may be distinct from tissue and the whole plant level.[2]

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[1]S. Kannan and S. H. Wittwer, "Effects of Chelation and Urea on Iron Absorption by Intact Leaves and Enzymically Isolated Leaf Cells," Plant Physiology Supplement, CXL (August, 1965), 12.

[2]W. H. Jyung and others, "Mode of Action of Some Growth Retardants in Tobacco Leaf Cells," Plant Physiology Supplement, XL (August, 1965), 13.

Many persons in the course of their studies, have suggested, but never proved, the possible coupling of ion absorption with different phases of metabolism at the cellular level. [1,2,3] Some have even gone so far as to suggest the possible correlation between ion absorption and respiration.[4]

Others have suggested that carriers are responsible for absorption of ions and that these carriers are probably proteinaceous in nature.[5,6]

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[1] S. H. Wittwer and F. G. Teubner, "Foliar Absorption of Mineral Nutrients," Annual Review of Plant Physiology, X (1959), 13-27.

[2] Jyung and Wittwer, op. cit., 442.

[3] R. C. Smith and E. Epstein, "Ion Absorption by Shoot Tissues; Kinetics of Potassium and Rubidium Absorption by Corn Leaf Tissue," Plant Physiology, XXXIX (1964), 992-996.

[4] W. H. Jyung, S. H. Wittwer and M. J. Bukovac, "Ion Uptake by Cells Enzymically Isolated from Green Tobacco Leaves," Plant Physiology, XXXX (May, 1965), 413.

[5] Jyung and Wittwer, op. cit., 443.

[6] W. H. Jyung and S. H. Wittwer, "Pathways and Mechanisms for Foliar Absorption of Mineral Nutrients," Agricultural Science Review (Second Quarter, 1965), 30.

# CHAPTER III

## MATERIALS AND METHODS

The cells used for the experiment were isolated from leaves of garden bean plants, Phaseolus vulgaris, sold under the brand name 105-green pod bush beans. Seeds for growing these bean plants were obtained from the Earl May Seed Store in Des Moines, Iowa.

To prevent fungal contamination, the seeds were soaked in a one per cent sodium hypochlorite (Chlorox Bleach) solution for fifteen minutes before planting. After soaking, the seeds were planted in large plastic trays, which contained approximately three inches of vermiculite. The seeds were planted approximately two inches below the surface of the vermiculite. The trays of seeds were placed on the rack of a tray stand, which had been completely enclosed by clear, transparent cellophane. A small hot plate heater was placed inside the enclosure to keep the temperature constant at approximately twenty-five degrees centigrade. Light was supplied by a special fluorescent tube (Sylvania Gro-lux, F15T8-Gro) placed inside near the top of the enclosure.

The plants were watered at regular intervals. One-half of the seeds were watered with a nutrient solution

containing all of the elements essential for normal plant growth. The remaining half was watered with a solution identical to the above except that phosphorus was not added to the mixture. A summary of the nutrient solutions used is given in Table I.

The vermiculite was kept nearly saturated until the seedlings had just begun to sprout. This took approximately five days. After the fifth day, nutrient solution was added every three or four days.

When the primary leaves of the seedlings, grown in the phosphate deficient solution, began to expand and were large enough to maintain the plant, the two cotyledons were removed. This reduced the amount of phosphate supplied by the cotyledons to the plants.

The primary leaves of the bean seedlings were excised when they reached approximately three-fourths of their fully expanded size. The seedlings were about two weeks old at this time and the leaves of the normal seedlings were slightly more than five centimeters in width, and the deficient leaves were only about three and one-half centimeters in width. After excising, the leaves were rinsed in water. With a small scissors, the midribs were removed and the remaining leaf sections were cut into narrow strips approximately two millimeters in width and two centimeters in length.

TABLE I

NUTRIENT SOLUTION USED AS GROWTH MEDIA FOR BEAN PLANTS[1]

| Chemical | Concentration | ml per four liters | |
|---|---|---|---|
| | | Complete | $PO_4$ deficient |
| $Ca(NO_3)_2$ | 0.25M | 20 | 20 |
| $KNO_3$ | 0.5M | 10 | 10 |
| $MgSO_4$ | 0.25M | 8 | 8 |
| Ferric Tartrate | 0.5% | 2 | 2 |
| $KH_2PO_4$ | 0.125M | 8 | -- |
| Trace Elements | * | 2 | 2 |

*Trace element solution (per liter): KCl, 1864mg; $H_3BO_3$, 773mg; $MnSO_4.H_2O$, 423mg; $ZnSO_4.7H_2O$, 200mg; $CuSO_4.5H_2O$, 63mg; $(NH_4)_6Mo_7O_{24}.4H_2O$, 9.2mg.

[1]E. H. Newcomb, G. C. Gerloff, and W. F. Whittingham, Plants in Perspective (San Francisco and London: W. H. Freeman and Company, 1964), p. 54.

Five grams of normal leaf strips were placed in each of two three hundred milliliter Erlenmeyer flasks, which contained seventy-five milliliters of a separating solution. The separating solution used is summarized in Table II. The same procedure was followed with the phosphate deficient leaf strips. Approximately ten grams of leaf strips was needed to obtain five grams fresh weight of isolated cells.

The flasks, containing the leaf strips in the separating solution, were placed in a constant temperature water bath shaker and shaken at one hundred and eighty excursions per minute for four hours at room temperature.

After four hours of shaking, the flasks were removed from the shaker and the contents filtered through four layers of cheese cloth, into a beaker. This removed any remaining parts of intact leaf strips and any large clumps of cells. This also removed most of the loose vascular material from the solution.

The filtrate was added to fifty milliliter centrifuge tubes and centrifuged at one hundred and seventy-five times the acceleration of gravity for two minutes. The green supernatant liquid was drawn off with a pipette. To the remaining cells was added twenty-five milliliters of a rinsing solution (Table III). After shaking the tubes

TABLE II

SEPARATION SOLUTION WITH PH ADJUSTED TO 6.4 AND FINAL VOLUME OF 500 MILLILITERS

| Chemical | Concentration of stock solution | Amount added to mixture | Final Concentration[1] |
|---|---|---|---|
| Glycerol | 10% | 10ml | 0.2% |
| Pectinase | -- | 1g | 0.2% |
| Peptone | -- | 1g | 0.2% |
| Sucrose | 0.5M | 100ml | 0.1M |
| EDTA(ph 6.4) | 0.1M | 100ml | 0.02M |
| Sodium Succinate | 0.1M | 50ml | 0.01M |
| Potassium Citrate | 0.1M | 50ml | 0.01M |
| Tris-maleate (ph 6.4) | 0.075M | 134ml | 0.02M |

TABLE III

RINSING SOLUTION WITH FINAL VOLUME OF 200 MILLILITERS

| Chemical | Concentration of stock solution | Amount added to mixture | Final Concentration[2] |
|---|---|---|---|
| Sucrose | 0.5M | 140ml | 0.35M |
| Calcium Chloride | 0.01M | 4ml | 0.0002M |

[1] Jyung, Wittwer, and Bukovac, loc. cit.

[2] Ibid.

lightly to dislodge the cells from the bottom of the tube, they were re-centrifuged for two minutes. This rinsing and re-centrifuging procedure was repeated until the supernatant liquid was colorless.

After the clear supernatant liquid had been removed from the last centrifugation, twenty-five milliliters of the incubation solution (Table IV) was added to the cells.

TABLE IV

INCUBATION SOLUTION WITH PH ADJUSTED TO 6.4 AND FINAL VOLUME OF 300 MILLILITERS

| Chemical | Concentration of stock solution | Amount added to mixture | Final concentration in micro moles per 5ml[1] |
|---|---|---|---|
| Sucrose | 0.5M | 70ml | 585 |
| Tris-maleate | 0.75M | 40ml | 50 |
| Socium Citrate | 0.1M | 6ml | 10 |
| Sodium Succinate | 0.1M | 6ml | 10 |
| $MgCl_2$ | 0.1M | 0.3ml | 0.5 |
| Tween-20 | 100% | 0.3ml | 0.01% |
| $KH_2PO_4$* | 0.05M | 0.6ml | 0.5 |

*$KH_2PO_4$ not added to controls

[1] Ibid.

After shaking lightly, the cells were again centrifuged at two hundred and fifty times the acceleration of gravity for five minutes. After the supernatant incubation solution had been drawn off, ten milliliters of concentrated cells were brought up to thirty five milliliters in volume with fresh incubation solution. The tubes were shaken lightly and stored in the refrigerator until needed.

The apparatus used for measuring the respiration rates of the cells was the Gilson Differential Respirometer. In preparing the respirometer for the experiment, the volumeters were each set on two hundred. The water bath was filled and the water temperature set at twenty-five degrees centigrade. The gassing valves, the operating valves and the disconnect valves were open and all connections were greased thoroughly with petroleum jelly (Vaseline) before connecting the respiratory flasks to begin the experiment.

The excess carbon dioxide given off during respiration was absorbed by five pellets of sodium hydroxide placed in the center wells of each of the fourteen flasks. After preparing the respirometer and the flasks, the tubes containing the suspended cells were removed from the refrigerator and shaken lightly to mix the cells evenly in the solution.

Five milliliters of the suspended normal cells was added to each of seven flasks of the respirometer. To each

of the remaining seven flasks was added five milliliters of suspended phosphate deficient cells. Six tenths of a milliliter of five-hundredths molar potassium hydrogen phosphate was added to four flasks each of normal and four flasks each of deficient cells. Three flasks, with only the suspended cells, remained as the control for both groups.

The reference flasks and the flasks containing the cells were then attached to the manometers and lowered into the water bath. After turning on the shaker, the cells were left to rock for fifteen minutes with all the valves still open. This allowed the cells time to come to an equilibrium with the respirometer system.

After the cells had reached an equilibrium, the gassing valve and the operating valves were then closed and volumes of oxygen consumed per gram fresh weight were measured every ten minutes for two hours and ten minutes in run one and one hour and ten minutes in run two.

# CHAPTER IV

## RESULTS AND INTERPRETATION OF DATA

Twenty normal and thirty phosphate deficient plants were used for the isolation procedure. Growth characteristics differed considerably between plants grown in complete nutrient media and plants grown in phosphate deficient media.

Plants in the deficient media grew at a slower rate than did the normal plants. The deficient plants were much shorter than the normal plants. The leaves were smaller and the more deficient leaves curled under around their borders and were a darker green in color. These growth characteristics are consistent with those reported for legumes by De Turk.[1]

Cells isolated from both normal and deficient leaves were examined under the microscope. The masses of cells were of palisade and spongy mesophyl origin. There were a few epidermal cells, guard cells and small aggregates of vascular tissue present. No physical differences in size or number of chloroplasts were observed.

Readings of oxygen consumption were taken from each

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[1] C. E. De Turk, Plant Nutrient Deficiency Symptoms in Legumes (Chapter VIII of Hunger Signs in Crops, ed. Grove Hambridge. Washington: The American Society of Agronomy and the National Fertilizer Association, 1941), p. 247.

of the fourteen flasks at ten minute intervals. The observations recorded in Table V are the averages of each group.

The respiratory trends for both the normal and deficient cells with phosphate added and their controls were similar (Figure 1). In all four groups the respiratory activity was the lowest during the first thirty minutes of the experiment. During this time the cells had not reached equilibrium with the atmosphere of the respirometer.

After the first thirty minutes, a noticeable increase in oxygen consumption of all four groups of cells was observed. This increase lasted for approximately fifty minutes, after which the cells activity slowly decreased. An exception to this trend was in the group of flasks containing the phosphate deficient grown cells with the phosphate added. The respiratory activity of these cells continued at a more rapid rate until the termination of the experiment. Evidence as to this point may be observed in the straight line expansion of the phosphate deficient cells with phosphate added, as seen in Figure 1.

Although the respiratory trends of each group of cells was similar, there were significant individual rate differences between the variables in this experiment. The most pronounced difference in activity occurred between the

TABLE V

AVERAGES OF OXYGEN UPTAKE READINGS TAKEN AT TEN MINUTE INTERVALS FROM THE GILSON RESPIROMETER

| Time in Minutes | Oxygen Uptake in Micro liters | | | |
|---|---|---|---|---|
| | Normal Cells | | Deficient Cells | |
| | Control | $PO_4$ Added | Control | $PO_4$ Added |
| 10 | 4.6 | 5.7 | 5.7 | 1.1 |
| 20 | 8.2 | 8.6 | 9.2 | 6.2 |
| 30 | 12.9 | 14.0 | 13.3 | 12.9 |
| 40 | 18.6 | 21.3 | 19.0 | 22.8 |
| 50 | 25.5 | 28.5 | 26.5 | 34.1 |
| 60 | 34.7 | 40.4 | 35.1 | 45.9 |
| 70 | 47.3 | 51.3 | 46.1 | 59.2 |
| 80 | 55.2 | 60.7 | 56.3 | 71.2 |
| 90 | 65.3 | 69.4 | 65.3 | 82.8 |
| 100 | 73.1 | 77.1 | 73.4 | 93.3 |
| 110 | 81.7 | 84.2 | 82.1 | 102.9 |
| 120 | 85.3 | 91.8 | 89.6 | 114.4 |
| 130 | 93.5 | 97.6 | 98.0 | 125.5 |

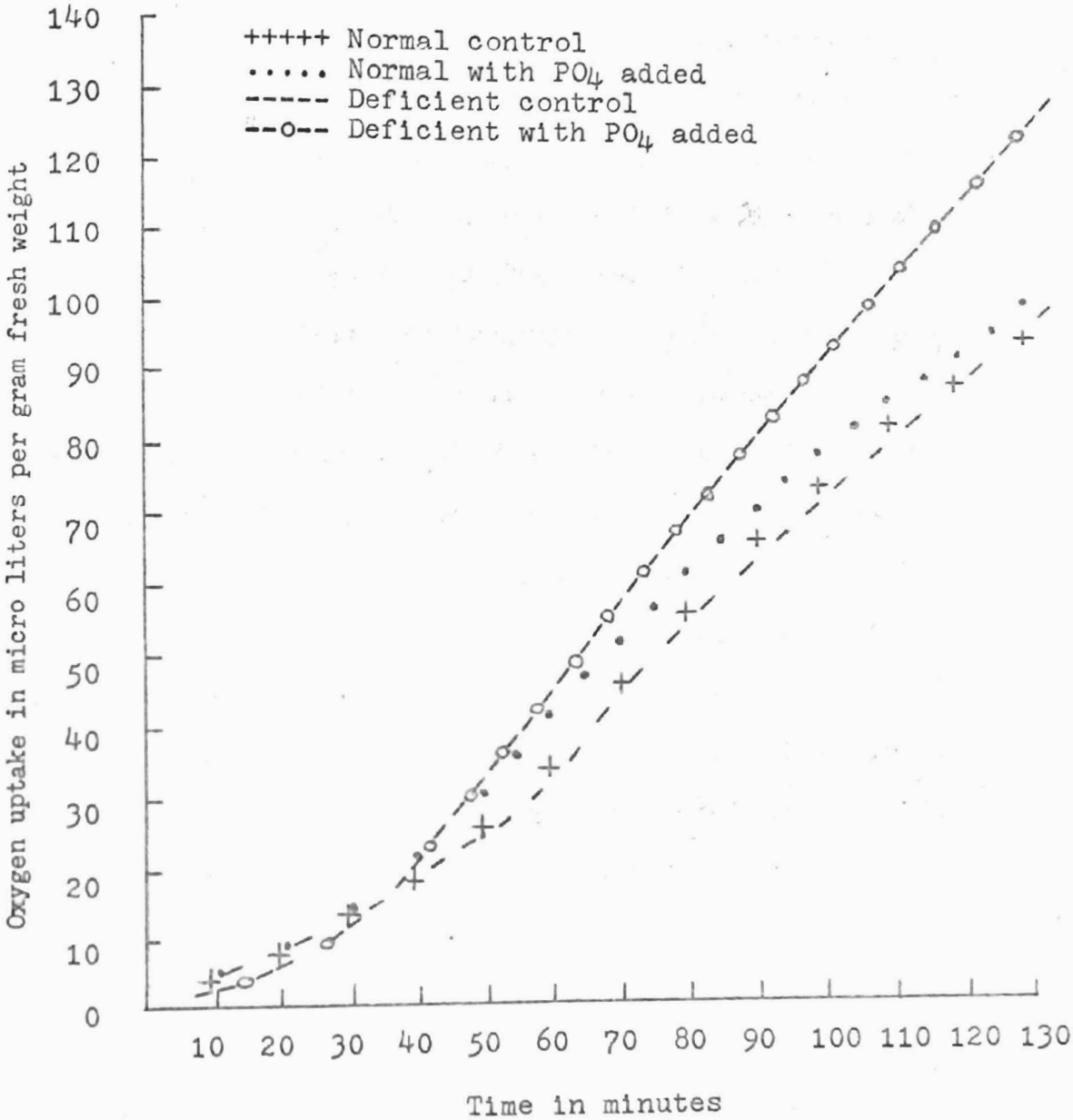


Figure 1. Consumption of Oxygen by two week old isolated leaf cells of Phaseolus vulgaris.

phosphate deficient cells to which phosphate had been added and the deficient control. After complete quilibrium was reached, the rate of oxygen consumption of the deficient cells to which phosphate had been added rapidly increased over the phosphate deficient cells control. The respiratory activity of phosphate enhanced cells did not decline. The total oxygen consumed of phosphate enhanced cells continued to increase over the control for the remainder of the experiment. This increase in activity over the control was significant (t test) at the .08 probability level.

The difference in activity of the normal grown cells with phosphate added and the normal controls was not as pronounced. For a period of thirty minutes after equilibrium was reached, a slight increase in the activity of these phosphate enhanced cells occurred over the control cells. Unlike the deficient cells, the respiratory activity did not continue to increase, but leveled off and remained at nearly the same rate as the controls for the remainder of the experiment. This increase in activity was not statistically significant (t test).

The cause of the large increase in activity of the deficient cells and the brief increase in activity of the normal cells was associated with the phosphate added to the incubation mixture.

Phosphorus plays a key role in many of the metabolic processes of the cell such as respiration, photosynthesis and energy transfer. Phosphorus, as adenosine triphosphate (ATP), is involved in practically every synthetic reaction of the cell. A deficiency of phosphate in the cell would suppress the formation of ATP and other phosphorylated compounds, whether derived from photosynthetic or oxidative phosphorylation.[1] Indirectly, protein synthesis, which is dependent upon ATP, would be affected. Nuclear activity and cell division would be suppressed due to the decreased synthesis of DNA and RNA.[2] All of these factors stemming directly from the lack of phosphorus would cause deficient growth characteristics previously described for the phosphate deficient plants.

The respiratory activity of the deficient cells without phosphate added was nearly equal to that of the normal cells without phosphate added. The deficient plants seem to have adapted themselves for maintaining a normal or near normal respiratory rate in the leaves. Nason and McElroy[3] report that under deficient conditions there is a redistribution of phosphorus within the plant whereby

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[1] A. Nason and W. D. McElroy, Modes of Action of the Essential Mineral Elements (in Vol. III of Plant Physiology, ed. F. C. Steward. New York: Acedemic Press, 1963), p. 148.

[2] Ibid.

[3] Ibid., pp. 508-509.

phosphorus is withdrawn from metabolic less active areas to cells in younger more active areas. The deficient conditions may have stimulated the redistribution of phosphorus from less active areas, such as the root or the stem, to the leaf, which is the area of greatest metabolic activity in a plant. In this way the deficient plants could maintain normal respiratory activity as long as the phosphorus supply lasted.

Eventually, with no phosphate entering the plant through either the roots or the leaves, the supply from other parts of the plant would soon be depleted and the respiratory activity of the leaves would begin to decrease. This effect is shown, to some extent, in the second replication of the experiment which is discussed later.

The plants used in the first run were not of a stage where the phosphorus supply from other parts of the plant had been depleted to the point of limiting respiration in the leaves. Deficient growth characteristics gave evidence that it was limiting in cells of leaf margins.

With the addition of phosphate to the deficient cells, the metabolic activity of these cells increased greatly over the control. This increase is probably due to the availability of phosphate and its utilization in the cell. Sutcliff reports that phosphate absorbed into barley roots is rapidly transferred into ATP and later appears in

hexose phosphate found in the respiratory cycles.[1] It has also been shown that $P^{32}$ can be recovered in hexose phosphate fractions after foliar application to tomato plant leaves.[2] If this is the case in bean leaves, then the phosphate added to the deficient cells could be utilized immediately in the formation of ATP, or it could enter directly into respiratory cycles. Either pathway would give rise to an increased respiratory rate.

Jennings reports that respiration in a cell may be governed by the cellular concentration of high energy phosphate acceptors, which in turn are determined by the activity of the various mechanisms causing hydrolysis of high energy compounds such as ATP.[3] Uptake of phosphate would increase the possibility of producing ATP in phosphorus starved cells.[4] Since phosphate is essential for formation of ATP, the lack of it could depress hydrolysis of ATP and thus decrease the respiration rate.

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[1] J. F. Sutcliff, Mineral Salt Absorption in Plants (New York: Pergamon Press, 1962), p. 88.

[2] F. G. Teubner and others, "Some Factors Affecting Absorption and Transport of Foliar Applied Nutrients as Revealed by Radioactive Isotopes," Quarterly Bulletin of Michigan Agricultural Experiment Station, XXIX (February, 1957), 389-415.

[3] D. H. Jennings, The Absorption of Solutes by Plant Cells (Ames, Iowa: Iowa State University Press, 1963), p. 72.

[4] A. Kylin, "Uptake and Loss of $Na^+$, $Rb^+$, and $Cs^+$ in Relation to an Active Mechanism for Extrusion of $Na^+$ in Scenedesmus," Plant Physiology, XXXXI (April, 1966), 584.

One of the mechanisms very likely necessary for hydrolysis of high energy compounds is active transport.[1] Cellular absorption of phosphate has been shown to be an active transport process.[2] Energy must be supplied for the transport mechanism to be effective. The usual energy source identified is ATP, which is supplied through the respiratory cycles or through photosynthetic phosphorylation.[3] Therefore a deficiency of phosphate could suppress ATP formation and suppress the active transport mechanism which in turn may decrease the respiration rate in the plant cells.

With the addition of phosphate to the normal cells, a slight initial increase in the respiratory rate occurred, after which the rate decreased and remained the same as the control for the remainder of the experiment. This increase may have been due to the phosphate added. In these normal cells the phosphate was not present in sufficient quantities to cause a maximum respiratory rate. When the phosphate was added, it could have caused the rate to increase to a maximum, for a short period of time, through mechanisms previously described for deficient cells.

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[1] Jennings, loc. cit.

[2] W. H. Jyung, and S. H. Wittwer, "Foliar Absorption - An Active Uptake Process," American Journal of Botany, LI (April, 1964), 437-444.

[3] W. H. Jyung and S. H. Wittwer, "Pathways and Mechanisms for Foliar Absorption of Mineral Nutrients," Agricultural Science Review (Second Quarter, 1965), 33.

A second series of observations was performed to verify the results of the first. Plants used for the isolation procedure in this run were one week older than the plants used previously. The results obtained were similar. There was an increase of the normal and deficient cells activity over the controls. The deficient cells increase was greater than the normal cells.

The respiratory activity, as determined by oxygen consumption, was less than for cells in the first series of determinations. A primary cause for this was the increased age of the plants used. The maximum respiration rate for leaves is reached about seven days after sprouting. After approximately seven days there is an immediate sharp decline in activity.[1] Plants used in the first run were two to three days past this period of maximum activity. The rates of these older plants were, as expected, somewhat lower than those reported for whole leaves of other plants.[2]

Plants used in the second series of determinations were about fifteen days past the period of maximum activity and well into the period of sharp decline. This could account for the lower rates in the second experiment.

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[1]E. W. Yemm, Respiration of Plants and Their Organs (in Vol. IVA of Plant Physiology, ed. F. C. Steward. New York: Acedemic Press, 1965), p. 270.

[2]J. Bonner and A. W. Galston, Principles of Plant Physiology (San Francisco: W. H. Freeman and Company, 1952), p. 223.

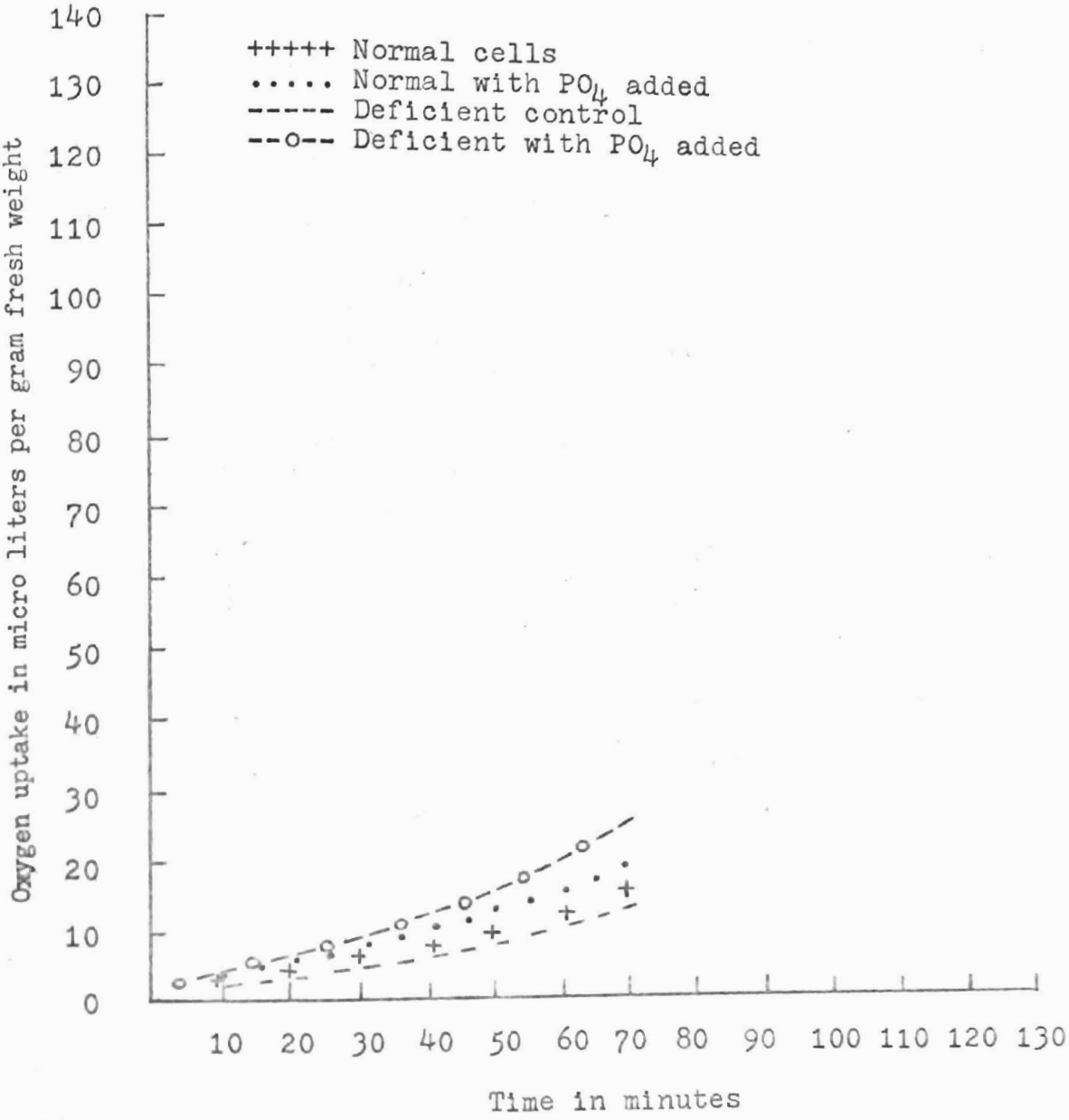


Figure 2. Consumption of oxygen by three week old isolated leaf cells of Phaseolus vulgaris.

In series one the experiment was terminated after one hundred and thirty minutes and in series two after only seventy minutes. The respiratory activity of the cells after these times decreased sharply, approaching zero approximately fifteen minutes after the termination times. Age and temperature effects in the isolation procedure were the most likely causes for the early decline in cell activity. The isolation procedure was carried out at room temperature. Under ideal conditions, the whole procedure, up to transferring cells to the respiration flasks on the respirometer, would be carried out in a room with the temperature set at four degrees centigrade. Under these preliminary conditions, the cells likely would have remained active for a longer period of time and a greater amount of data with more significant results might have been obtained.

From the results of the experiment performed and the literature cited, it appears that respiration is associated with phosphate uptake in plant leaf cells. A similar correlation has been shown in certain roots.[1] The relationship could be direct, with phosphate entering directly into the respiratory cycle as hexose phosphates. It could be an indirect relationship with the phosphate comining to form ATP. In either pathway it would cause an increase in

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[1]Jennings, op. cit., p. 68.

respiratory activity up to the maximum rate where phosphate is no longer a limiting factor. For this increase in respiratory activity, phosphate must incorporate into ATP. ATP is needed as a source of energy for active transport of phosphate into the cell. Therefore phosphate is essential in the metabolic process.

There are still many questions left unanswered to be correlated by future experimentation. Phosphate is likely correlated with other mechanisms and other phases of the metabolic process such as protein synthesis. Isolation of these mechanisms and processes and their correlation with metabolism will provide the basis for much future work.

# CHAPTER V

## SUMMARY

In recent years, an increasing emphasis has been placed on studies of pathways and mechanisms for entry of ions into the leaf. Most studies of ion absorption in leaves have been done upon isolated cells.

A number of investigations have given indirect evidence of a correlation between foliar ion uptake and respiration in the leaf. It was the purpose of this investigation to observe if any correlation occurred between uptake of the phosphate ion and oxygen consumption in isolated bean (Phaseolus vulgaris) leaf cells.

Plants used for this experiment were grown under greenhouse conditions. One-half were grown in a phosphate deficient medium. The other half were grown in a complete nutrient medium. Leaves were excised from the plant and added to a solution which enzymically separated cells from the surrounding tissue. The cells were filtered and centrifuged until primarily palisade and spongy mesophyl cells remained suspended in the solution. These cells were added to an incubation solution and placed in flasks of the Gilson Differential Respirometer. One-half of the fourteen flasks contained normal cells. The other seven flasks

contained phosphate deficient cells. Six tenths milliliter of five hundredths molar potassium hydrogen phosphate was added to each of four flasks in each group. Oxygen uptake per gram fresh weight of cells was measured in micro liters and recorded for each flask.

Cells from both the normal and deficient phosphate grown bean plants, when phosphate was added, consumed oxygen at a more rapid rate than did their controls, which had no phosphate added. The increase in oxygen consumption of the normal cells, to which phosphate was added, was small and occurred only for a short time. The increase in oxygen consumption of the deficient cells, to which phosphate was added, was significantly greater and continued throughout the experiment.

It was concluded that phosphate ion uptake by leaf cells of beans was correlated with increased oxygen consumption. This correlation could be either direct, by combining to form hexose phosphates, or indirect through incorporation into the adenosine triphosphate molecule, which in turn may provide a source of energy for the active transport of more phosphate into the cell.

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