Plant Physiol. (1972) 49, 436–440

Increased Membrane-bound Adenosine Triphosphatase Activity Accompanying Development of Enhanced Solute Uptake in Washed Corn Root Tissue[1]

Received for publication August 9, 1971

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ABSTRACT

Washing of excised corn (*Zea mays* L., variety WF9xM14) root tissue is accompanied by an increase in (Mg^{2+} + K^+)-stimulated adenosine triphosphatase. This is the adenosine triphosphatase described by Fisher, Hansen, and Hodges as positively correlated with ion accumulation rates. The increase in activity is confined to the microsomal fraction. A close parallel exists between increases in adenosine triphosphatase and phosphate absorption, and they respond similarly to inhibitors of RNA and protein synthesis. However, the amplitude of change is much smaller in adenosine triphosphatase. Possible reasons for this discrepancy are discussed.

In the preceding paper (25) we describe the increase in absorption rates of phosphate, and of some other ions and solutes, which accompanies the washing of roots from seedlings grown under highly aerobic conditions. After an inductive lag period of about 30 min, there is a rapid development of phosphate absorption rate without significant change in respiration rate. Inhibitor studies indicate that RNA and protein synthesis are involved. However, no increase in membranes with washing could be found by electron microscopy or phospholipid analysis. Therefore, "transport" enzymes synthesized must be added to existing membranes. Since the apparent K*m* values for ^{32}P and ^{86}Rb absorption do not change, it would appear that existing carrier systems must be augmented or activated.

One enzymatic system which is being explored for a possible role in ion transport is membrane-bound ATPase (1, 4, 5, 7–10, 12, 15, 17, 22, 29, 33). Fisher *et al.* (10) recently reported a high correlation between a Mg^{2+}-requiring, Rb^+-stimulated ATPase and Rb^+ influx rates. We report here that the K^+-stimulated ATPase from the microsomal fraction of corn roots increases in parallel with phosphate absorption rates induced during washing of root tissue.

MATERIALS AND METHODS

The 0.5- to 2.5-cm section behind the tip of the primary root of 3-day-etiolated corn seedlings (*Zea mays* L., variety WF9 × M14) was used throughout this study. Seedlings were grown on toweling as described in the accompanying paper (25). Inductive washing was by immersion of sections in well aerated 0.2 mM $CaCl_2$ solution. Increased transport rates were assessed by measuring rates of ^{32}P-labeled phosphate absorption from 0.2 mM $CaCl_2$ + 0.2 mM potassium phosphate, pH 6. Both procedures are described in the preceding paper (25).

Incorporation and Distribution of ^{14}C-Leucine. Root segments were incubated in 500-mg lots for 15 min in 10 ml of 0.2 mM $CaCl_2$ containing 0.1 μc/ml of uniformly labeled ^{14}C-leucine (316 mc/mmole). After being rinsed for 30 sec in ice-cold water and for 20 min in ice-cold unlabeled 1 mM leucine, the tissue was ground with a conical glass homogenizer in 0.2 M sucrose. The homogenate was successively centrifuged at 2,000*g* for 10 min (cell wall and nuclei), 12,000*g* for 20 min (mitochondria), and 80,000*g* for 60 min. The pellets were washed by resuspension and recentrifugation and were suspended in 0.2 M sucrose. Protein was precipitated from the supernatant, first in 50% saturated $(NH_4)_2SO_4$ and finally in 8% trichloroacetic acid. Each fraction was washed by resuspension and centrifugation and finally was suspended in 0.2 M sucrose. Aliquots of the pellets, precipitates, and final supernatant were assayed for radioactivity. Protein content was determined colorimetrically (27).

Incorporation and Distribution of ^{32}P. Root segments were incubated for 15 min in 30 ml of aerated 0.2 mM $CaCl_2$ plus 5 μM potassium phosphate, pH 6.0, labeled with 0.05 μc/ml carrier-free ^{32}P. After being rinsed for 30 sec in ice-cold water and for 30 min in ice-cold 2 mM potassium phosphate, pH 6.0, the weighed tissue was homogenized with cold 0.2 N perchloric acid in a conical glass homogenizer. The phosphate-containing fractions were extracted by the procedures of Hall and Hodges (16). Organic and inorganic phosphate of the acid-soluble fraction were separated by the isobutanol-benzene extraction of phosphomolybdate (30), and total inorganic phosphate was determined, as well as radioactivity. Radioactivity of all fractions was determined by scintillation counting.

ATPase Determinations. Root segments were ground with a chilled mortar and pestle in 0.25 M sucrose plus 3 mM EDTA adjusted to pH 7.8 with tris. The homogenate was strained through cheesecloth and used directly, or successively centrifuged at 1,500*g* for 10 min (cell wall-nuclei), 12,000*g* for 20 min (mitochondria), and 80,000*g* for 60 min, or 80,000*g* for 60 min through a 0.4 M sucrose wash layer (microsomes). The supernatant was diluted with 0.25 M sucrose to reduce the EDTA to 1 mM, and the washed particulate fractions were suspended in 0.25 M sucrose plus 1 mM EDTA.

In later stages of experimentation, ATPase was assayed

[1] Research was supported by a grant from the Atomic Energy Commission (AT 11-1-790) and a United States Public Health Service predoctoral traineeship to R. T. L.

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Table I. *Effect of Washing on the Distribution of ^{14}C-Labeled Leucine in Various Cell Fractions of Corn Root Segments*

Forty root segments were exposed to ^{14}C-leucine for 15 min before and after washing for 105 min as indicated. See "Materials and Methods" for details.

Fraction	% of Total Recovered			% of Total Incorporated/mg Protein		
	Fresh control	Washed	Washed in 0.1 mM 2,4-D	Fresh control	Washed	Washed in 0.1 mM 2,4-D
Cell wall-nuclear	3.9	9.6	12.7	9.1	18.1	26.0
Mitochondrial	5.4	5.2	4.7	40.8	33.6	30.7
Microsomal	4.2	4.0	3.7	31.9	27.1	22.3
50% NH_4SO_4 ppt	2.1	2.3	2.3	7.4	9.8	9.7
8.2% trichloroacetic acid ppt	5.4	8.1	8.6	10.8	11.5	11.2
Soluble	79.1	70.7	68.0			
Total cpm recovered	159,113	465,561	349,922	71,565	277,506	215,413

on the microsome fraction only. Exploratory work showed that a fraction sedimenting between 12,000*g* for 20 min and 46,000*g* for 30 min had the highest increase in specific activity due to washing and was thus used as the microsome fraction.

ATPase activity was assayed by measuring the release of inorganic phosphate from ATP, according to the method of Fisher *et al.* (10). The final reaction volume was 1.0 ml and consisted of 3 mM ATP (tris salt, pH 6.8), 1.5 mM $MgCl_2$ when added, 50 mM KCl when added, and from 50 to 100 μg of protein. The cell wall and mitochondrial fractions were assayed at their pH optimum, pH 8.0 (9). After a 30-min incubation period at 38 C in a shaking water bath, the reaction was terminated by the addition of 1.0 ml of cold 10 N H_2SO_4-5% ammonium molybdate-H_2O (2:2:1). The inorganic phosphate content was measured by the method of Fiske and Subbarow (11). Protein was determined according to the method of Lowry *et al.* (27).

The microsomal fraction collected for ATPase determination was assayed for NADH-cytochrome c reductase by following the reduction of cytochrome c at 550 nm. The final reaction volume was 3.0 ml and consisted of 25 μM NADH, 25 μM cytochrome c, 0.167 mM KCN, and 50 mM KH_2PO_4, pH 7.6. The reaction was run at room temperature, about 24 C. Enzyme concentration was chosen to give an optical density change of less than 0.1 unit/min (about 20 μg protein). The reaction was initiated by the addition of substrate, and absorbance was recorded at 15-sec intervals for 2 min. The extinction coefficient of cytochrome c was taken as 18.5 mM^{-1} cm^{-1} at 550 nm.

RESULTS

Labeling Studies. An investigation was made of ^{14}C-leucine and ^{32}P incorporation to determine if membranous fractions were preferentially labeled as a result of washing. After 1.75 hr of washing, when the rate of increase in absorption rate was maximal (25), the tissue was exposed to ^{14}C-leucine or ^{32}P for 15 min and then rinsed and homogenized, and the homogenate was fractionated. Two controls were used; freshly cut tissue and tissue washed a like period but in solution containing 0.1 mM 2,4-D to suppress the enhancement of absorption rates. Results are given in Tables I and II.

Washing increased both the rate of leucine absorption and percentage incorporation, but actually decreased the proportionate incorporation into the mitochondrial or microsomal fractions where one might expect to find fragments of plasmalemma. The only dramatic increase with washing lay in the relative incorporation into the cell wall-nuclear fraction.

Table II. *Effect of Washing on ^{32}P Incorporation into Corn Root Segments*

Root segments were exposed to ^{32}P-labeled phosphate for 15 min before and after washing for 105 min as indicated. See "Materials and Methods" for details.

Fraction	% of Total Absorbed		
	Fresh control	Washed	Washed in 0.1 mM 2,4-D
Lipid	2.2	1.8	2.5
Nucleic acid	3.4	2.6	8.5
Protein	0.7	0.5	1.0
Residue	0.3	0.2	0.8
Acid soluble			
Organic	86.8	86.1	79.3
Inorganic	6.6	8.7	7.9
Total ^{32}P absorbed (nmoles/g fresh wt·15 min)	6.7	19.1	11.8
P_i in tissue (μmoles/g fresh wt)	2.98	2.95	3.08

Increase here was largely at the expense of the acid-soluble fraction which contains the free amino acid.

Addition of 2,4-D depressed the increase in absorption rate but still further increased the labeling of the cell wall-nuclear fraction. Hence it is not clear that biosynthetic events in this fraction have much to do with the increase in transport upon washing.

Relative incorporation of ^{32}P into the lipid fraction was not much altered by washing (Table II). As with the ^{14}C-leucine pulse labeling, there is no evidence for major change in turnover of membrane constituents due to washing. There was a trend for more of the entering ^{32}P to enter the inorganic phosphate pool, but this was only a small fraction of the total absorbed. No change in the concentration of inorganic phosphate resulted from washing, and it appears unlikely that the pulse of $^{32}P_i$ equilibrated significantly with the inorganic pools, since more than 90% of the pulse label appeared in organic combination. The relatively trivial amount of entering $^{32}P_i$ was caught up in cytoplasmic phosphate turnover, with less than 10% transferred to the major inorganic phosphate pool, presumably vacuolar.

Again, 2,4-D during washing suppressed the development of increased transport rates, and this was accompanied by a general increase of ^{32}P labeling of cytoplasmic constituents at the expense of soluble organic phosphates. As expected

from previous work (21), the nucleic acids showed the greatest relative increase. The proportion of label transferred to the inorganic phosphate pools was slightly reduced.

ATPase Studies. It was evident from the labeling studies that no pronounced turnover of membrane proteins or phospholipids is involved in the washing response. Perhaps none should be expected; a critical transport enzyme might well be increased in quantity or activity without major changes in membrane constituents. What is needed is direct determination of the activity of some enzymes known to be linked to solute absorption. No such enzyme is known, but, as pointed out above, membrane-bound ATPase requiring Mg^{2+} and K^+ shows high correlation with ion absorption rates (10). We examined the activity of this enzyme for changes during tissue washing.

Exploratory experiments with homogenates showed tissue washing to produce higher ATPase both with and without added K^+ (Table III). Washing at ice temperature or with cycloheximide or 6-methylpurine prevented the increase.

Centrifugal fractionation of the homogenates localized the Mg^{2+}-dependent, K^+-stimulated ATPase in the particulate fractions (Table IV). The ATPase of the soluble fraction was inhibited by Mg^{2+}, and, although 50 mM K^+ enhanced activity, this was probably due to displacement of Mg^{2+} from the inhibitory sites, since activity never rose above that without added ions. Thus the high level of soluble ATPase did not exhibit properties of interest for this work.

In the particulate fractions, we confirmed the observation of Fisher and Hodges (8) that K^+ does not increase activity unless Mg^{2+} is present (unreported). Only the microsomal fraction showed an increase in K^+-stimulated ATPase due to washing at 30 C (Table IV). The mitochondrial ATPase declined.

The increase in microsomal ATPase activity due to washing was small (Table IV). However, statistical analysis of data from 12 experiments comparing microsome fractions from fresh tissue and tissue washed for 3 hr shows that the increase in ATPase activity is significant (Table V). Mg^{2+}-requiring, K^+-stimulated ATPase activity showed the highest percentage increase due to washing. ATPase activity with Mg^{2+} alone or without added ions also increased significantly as a result of washing. Thus even in a limited assay system the enzyme showed augmented activity. Since we want to follow that aspect of ATPase activity which is established to correlate with uptake (10), primary attention was given in subsequent work to the K^+-stimulated activity.

Microsomes can be expected to contain a heterogeneous mixture of membranous vesicles derived from fragmented endoplasmic reticulum, dictyosomes, tonoplast, plasmalemma, mitochondria, and plastids. In order to determine if there was a general rise in membrane-linked activity rather than a specific increase in K^+-stimulated ATPase, changes in the ATPase and NADH-cytochrome c reductase were compared in time (Fig. 1). The latter enzyme is cytochrome b_5 linked in animal tissues and is variously attributed to endoplasmic reticulum

Table III. *Effect of Various Washing Treatments on K^+-Stimulated ATPase Activity of Homogenates of Corn Root Tissue*

Homogenates prepared from segments were washed as indicated. See "Materials and Methods" for details. Homogenates were diluted to give approximately 60 μg protein per assay, which contained 1.5 mM $MgCl_2$; 0.6 mM tris-ATP, pH 6.5; and 50 mM KCl (when added).

Washing Pretreatment	ATPase Activity		
	$-K^+$	$+K^+$	Difference (K^+ stimulation)
	μmoles P_i/mg protein·hr		
None	20.15	21.77	1.62
3 hr, 30 C	22.63	24.76	2.13
3 hr, 2 C	20.86	22.43	1.57
3 hr, 30 C + cycloheximide (10 μg/ml)	20.01	21.67	1.66
3 hr, 30 C + 6-methylpurine (0.5 mM)	20.60	22.21	1.61
None	18.33	19.70	1.37
1 hr, 30 C	18.90	20.44	1.54
2 hr, 30 C	20.01	21.86	1.85

Table IV. *ATPase Activity of Centrifugally Fractioned Corn Root Homogenates*

For fractionation procedure see "Materials and Methods." In this experiment, the fraction sedimenting between 12,000*g* for 20 min and 80,000*g* for 1 hr through 0.4 M sucrose wash layer is the microsomal fraction. Reaction mixture contained 1.5 mM $MgCl_2$ (when added), 50 mM KCl (when added), 3 mM tris-ATP, enzyme, and water to give a final volume of 1 ml.

Fraction	Ion Added	Washing Pretreatment	
		3 hr, 2 C	3 hr, 30 C
		μmoles P_i/mg protein·hr	
Cell wall-nuclear	None	3.49	4.03
	Mg^{2+}	4.60	5.00
	Mg^{2+} + K^+	5.82 (1.22)[1]	5.79 (0.79)
Mitochondrial	None	3.34	3.48
	Mg^{2+}	12.21	13.12
	Mg^{2+} + K^+	17.35 (5.14)	16.85 (3.73)
Microsomal	None	3.29	3.57
	Mg^{2+}	6.91	8.17
	Mg^{2+} + K^+	9.01 (2.10)	10.77 (2.61)
Soluble	None	47.32	51.66
	Mg^{2+}	45.42	48.29
	Mg^{2+} + K^+	45.42 (0.00)	50.23 (1.94)

[1] Figures in parentheses: differences between ATPase activity with Mg^{2+} + K^+ and with Mg^{2+} alone.

Table V. *Microsomal ATPase Activity of Corn Root Tissue*

Numbers in table are means of 12 experiments comparing microsome fractions from fresh tissue washed in 0.2 mM $CaCl_2$ at 30 C for 3 hr.

Ion Added	Fresh	Washed	Difference, Washed − Fresh	Increase
	μmoles P_i/mg protein·hr			%
A. None	4.62	4.96	0.34 ± 0.14[1]	8
B. Mg^{2+}	9.67	11.07	1.40 ± 0.43[2]	14
C. Mg^{2+} + K^+	12.74	15.01	2.27 ± 0.53[2]	18
B − A. Mg^{2+} stimulation	5.05	6.11	1.06 ± 0.36[1]	21
C − B. K^+ stimulation	3.07	3.94	0.87 ± 0.18[2]	27

[1] Difference is significant at 5% level.
[2] Difference is significant at 1% level.

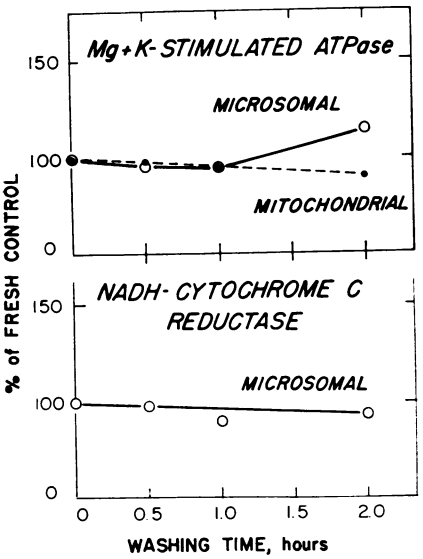

FIG. 1. Effect of washing time on ion-stimulated ATPase activity of mitochondrial (12,000*g* for 20 min) and microsomal (80,000*g* for 1 hr) fractions and on microsomal NADH-cytochrome c reductase activity of corn root segments. ATPase assay performed as described in Table IV. NADH-cytochrome c reductase was determined by following the reduction of cytochrome c at 550 nm (see "Materials and Methods").

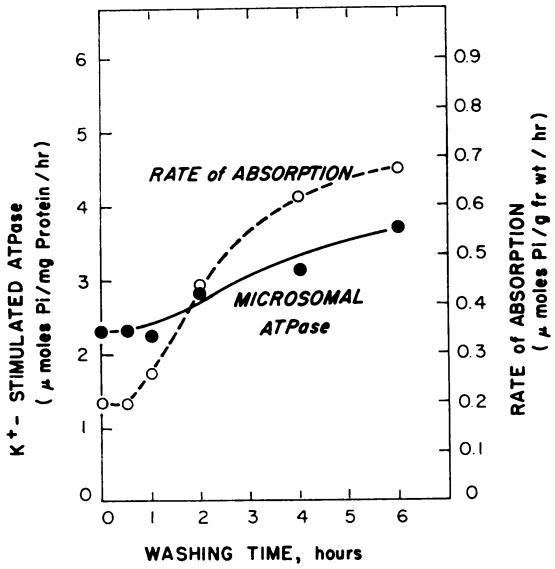

FIG. 2. Time course for the increase in Mg^{2+}-requiring, K^+-stimulated ATPase activity and in the rate of phosphate absorption during washing of corn root tissue. The microsomal fraction sedimenting between 12,000*g* for 20 min and 46,000*g* for 30 min was used for the ATPase assay, which was performed as described in Table IV.

and outer mitochondrial membrane (23). If there is an energy source alternative to ATP for respiration-linked ion uptake by plant membranes, it is conceivable that an NADH-oxidizing flavoprotein might be involved. However, only the microsomal ATPase increased, and this after a lag period of 1 hr.

A more detailed time course experiment was run to determine the degree of correlation between the development of K^+-stimulated ATPase of the microsomes and increased transport capacity (Fig. 2). There is a correlation, but the amplitudes of response to washing are quite different. If the induction and development of K^+-stimulated microsomal ATPase underlie the enhanced transport capacity, there may be, in addition, development of some unknown factor amplifying enzyme activity.

A further correlative test was made by comparing the washing response in the presence of inhibitors of RNA and protein synthesis (Table VI). There was good correlation between the increase in phosphate absorption and K^+-stimulated ATPase, but again the amplitude of the latter response is smaller. It is of interest that the correlation extended to the negative response to cycloheximide.

Nucleotide specificity of the microsomal fraction was checked. Only ATPase was enhanced by washing. Phosphate release from CTP, GTP, and UTP declined 2, 5, and 9%, respectively, after 3 hr of washing at 30 C.

Table VI. *Effect of Inhibitors of RNA and Protein Synthesis on the Increase in Phosphate Absorption and on Microsomal ATPase Activity during Washing of Corn Root Segments*

Microsomes were sedimented between 12,000*g* for 20 min and 46,000*g* for 30 min. Fresh tissue absorbed 0.18 μmole P_i/g fresh wt·hr, and the K^+-stimulated ATPase of fresh tissue was 5.07 μmoles P_i released/mg protein·hr (see "Materials and Methods").

Washing Pretreatment	% Change from Fresh Tissue	
	Phosphate absorption	K^+-stimulated ATPase
3 hr, 30 C	122	29
+ actinomycin D (20 μg/ml)	0	6
+ 6 methylpurine (0.5 mM)	11	4
+ cycloheximide (10 μg/ml)	−39	−20

DISCUSSION

A major barrier to understanding the mechanism of active solute transport across cell membranes is identification of the energy supply and the membrane-bound enzymes that utilize it. The extensive work on the (Na^+ + K^+)-stimulated ATPase of animal membranes has led to a search for similar ATPase in plant membranes. Unfortunately, the specific Na^+ + K^+ pump and ATPase inhibitor, ouabain, is not an inhibitor of ion absorption in plants (7, 18), and an early report on the sensitivity of plant ATPases to ouabain (4, 5) has not been confirmed. Only in the case of halophytic plants has it been possible to isolate membrane preparations which show a (Na^+ + K^+)-stimulated ATPase (17, 22).

With nonhalophytes it has been possible to demonstrate salt-stimulated ATPases in homogenates (4, 5, 12, 17, 22) and centrifugal fractions thereof (7, 8, 12, 15, 29). Histochemical techniques have been used to localize ATPases at cell membranes (13, 14, 26, 28, 32), and correlations between salt-stimulated ATPase activity and ion absorption have been established (8–10). Support for the view that ATP levels govern ion absorption by roots is found in several reports (3, 6, 18–20, 24). However, Atkinson and Polya (2) and Polya and Atkinson (31) have found evidence to the contrary in beet root tissue. These workers demonstrated that the bulk of soluble ATPase is acid phosphatase (1), but they did not extensively investigate membrane-bound ATPase.

Our experiments add one more piece to the correlative evidence that ion-stimulated membrane-bound ATPase is implicated in salt transport. The evidence is strengthened because the correlation can be followed during the rapid induction and development of enhanced transport capacity and

during the inhibition of this development. Only the microsomal membrane fraction responds, and present work in another laboratory indicates that it may be possible to isolate the microsome component responsible (R. T. Leonard and T. K. Hodges, unpublished data).

However, correlations by no means establish that the ATPase functions in transport, and there are troublesome points to be cleared up. First, enhanced transport of several solutes can be obtained by washing the root tissue. (Ref. 25; *cf.* leucine and phosphate, this paper). If ATP is the energy source for transport, other ions and solutes in addition to K^+ might be expected to activate the enzyme. In this connection it should be pointed out that K^+ is not needed to demonstrate an enhanced ATPase with washing (Tables III and V).

There is also the disparity in the magnitude of response of transport capacity and microsomal ATPase to washing. Perhaps additional enzyme is only partially responsible for the large increase in transport; there may, in addition, be shifts in metabolism such that more ATP is available to the membrane sites. This could provide the needed amplification. However, there is no significant change in respiration (25) and, judging from leucine incorporation (Table I), no diminution in biosynthesis. An alternative is that only part of the K^+-stimulated ATPase represents transport enzyme associated with the plasmalemma, thus putting relative increases on a spuriously large base. Recent work in Hodges' laboratory supports this alternative: plasmalemma only represents a fraction of the total salt-stimulated microsomal ATPase (personal communication).

Questions of stoichiometry and specificity must abide some purification of that microsome component which increases in salt-stimulated ATPase activity during washing. Concurrently, the component must be traced to its cytological source. Should this prove to be plasmalemma the augmentation of ATPase with tissue washing could be of considerable value in studies of whether or not the enzyme has a function in membrane transport mechanisms.

LITERATURE CITED

1. Atkinson, M. R. and G. M. Polya. 1967. Salt-stimulated adenosine triphosphatase from carrot, beet and *Chara australis*. Aust. J. Biol. Sci. 20: 1069–1086.
2. Atkinson, M. R. and G. M. Polya. 1968. Effects of L-ethionine on adenosine triphosphate levels, respiration and salt accumulation in carrot xylem tissue. Aust. J. Biol. Sci. 21: 409–420.
3. Bledsoe, C., C. V. Cole, and C. Ross. 1969. Oligomycin inhibition of phosphate uptake and ATP labeling in excised maize roots. Plant Physiol. 44: 1040–1044.
4. Brown, H. D. and A. M. Altschul. 1964. Glycoside-sensitive ATPase from *Arachis hypogaea*. Biochem. Biophys. Res. Commun. 15: 473–483.
5. Brown, H. D., N. J. Neucere, A. M. Altschul, and W. J. Evans. 1965. Activity patterns of purfied ATPase from *Arachis hypogaea*. Life Sci. 4: 1439–1447.
6. Budd, K. and G. G. Laties. 1964. Ferricyanide-mediated transport of chloride by anaerobic corn roots. Plant Physiol. 39: 648–653.
7. Dodds, J. J. A. and R. J. Ellis. 1966. Cation-stimulated adenosine triphosphatase activity in plant cell walls. Biochem. J. 101: 31–32p.
8. Fisher, J. and T. K. Hodges. 1969. Monovalent ion stimulated adenosine triphosphatase from oat roots. Plant Physiol. 44: 385–395.
9. Fisher, J. D., D. Hansen, and T. K. Hodges. 1970. Correlation between Rb^+ influx into roots and RbCl stimulated ATPase. Plant Physiol. 46: S23.
10. Fisher, J. D., D. Hansen, and T. K. Hodges. 1970. Correlation between ion fluxes and ion-stimulated adenosine triphosphatase activity of plant roots. Plant Physiol. 46: 812–814.
11. Fiske, C. H. and Y. Subbarow. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66: 375–400.
12. Gruener, N. and J. Neumann. 1966. An ion-stimulated adenosine triphosphatase from bean roots. Physiol. Plant. 19: 678–682.
13. Hall, J. L. 1969. Localization of cell surface adenosine triphosphatase activity in maize roots. Planta 85: 105–107.
14. Hall, J. L. 1969. A histochemical study of adenosine triphosphatase and other nucleotide phosphatases in young root tips. Planta 89: 254–265.
15. Hall, J. L. and V. S. Butt. 1969. Adenosine triphosphatase activity in cell wall preparations and excised roots of barley. J. Exp. Bot. 20: 751–762.
16. Hall, J. R. and T. K. Hodges. 1966. Phosphorus metabolism of germinating oat seeds. Plant Physiol. 41: 1459–1464.
17. Hansson, G. and A. Kylin. 1969. ATPase activities in homogenates from sugarbeet roots, relation to Mg^{2+} and $(Na^+ + K^+)$-stimulation. Z. Pflanzenphysiol. 60: 270–275.
18. Hodges, T. K. 1966. Oligomycin inhibition of ion transport in plant roots. Nature 209: 425–426.
19. Jacoby, B. 1966. Influence of oligomycin on sodium and chloride uptake by bean root tissue. Plant Cell Physiol. 7: 307–311.
20. Jacoby, B. and D. E. Plessner. 1970. Oligomycin effect on ion absorption by excised barley roots and on their ATP content. Planta 90: 215–221.
21. Key, J. L. 1970. Hormones and nucleic acid metabolism. Annu. Rev. Plant Physiol. 20: 449–474.
22. Kylin, A. and R. Gee. 1970. Adenosine triphosphatase activities in leaves of the mangrove, *Avicennia nitida* Jacq. Influence of sodium to potassium ratios and salt concentrations. Plant Physiol. 45: 169–172.
23. Lardy, H. A. and S. M. Ferguson. 1969. Oxidative phosphorylation in mitochondria. Annu. Rev. Biochem. 38: 991–1034.
24. Larkum, A. W. D. and B. C. Loughman. 1969. Anaerobic phosphate uptake by barley plants. J. Exp. Bot. 20: 12–24.
25. Leonard, R. T. and J. B. Hanson. 1972. Induction and development of increased ion absorption in corn root tissue. Plant Physiol. 49: 430–435.
26. Lott, J. N. A. and T. K. Hodges. 1970. *In situ* localization of ATPase in plant roots. Plant Physiol. 46: S23.
27. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.
28. McClurkin, I. T. and D. C. McClurkin. 1967. Cytochemical demonstration of a sodium-activated and a potassium-activated adenosine triphosphatase in loblolly pine seedling root tips. Plant Physiol. 42: 1103–1110.
29. Neumann, J. and H. Gruener. 1967. A soluble ATPase from bean roots stimulated by monovalent cations. Israel J. Chem. 5: 107–116.
30. Penniall, R. 1966. Determination of Pi by the isobutanol-benzene extraction procedure. Anal. Chem. 14: 87–90.
31. Polya, G. M. and M. R. Atkinson. 1969. Evidence for a direct involvement of electron transport in the high-affinity ion accumulation system of aged beet parenchyma. Aust. J. Biol. Sci. 22: 573–584.
32. Sabnis, D. D., M. Gordon, and A. W. Galston. 1970. Localization of adenosine triphosphatase activity on the chloroplast envelope in tendrils of *Pisum sativum*. Plant Physiol. 45: 25–32.
33. Sexton, R. and J. F. Sutcliffe. 1969. Some observations on the characteristics and distribution of adenosine triphosphatases in young roots of *Pisum sativum* cultivar Alaska. Ann. Bot. (London) 33: 683–694.