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Phosphate Absorption Rates and Adenosine 5′-Triphosphate Concentrations in Corn Root Tissue[1, 2]

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ABSTRACT

The correlations between ATP concentration in corn (*Zea mays*) root tissue and the rate of phosphate absorption by the tissue have been examined. Experimental variation was secured with 2,4-dinitrophenol, oligomycin, mersalyl, L-ethionine, 2-deoxyglucose, N_2 gassing and inhibition of protein synthesis. It is concluded that ATP could be the energy source for potassium phosphate absorption, but only if the transport mechanism possesses certain properties: oligomycin-sensitivity; creation of a proton gradient susceptible to collapse by uncouplers; phosphate transport via a mersalyl-sensitive Pi^--OH^- transporter; good activity at energy charge as low as 0.4; short enzymatic half-life for the ATPase or phosphate transporter; a linked mechanism for K^+-H^+ exchange transport, possibly electrogenic.

In previous investigations of the enhanced solute absorption rates which develop in washed corn root tissue, Leonard and Hanson (25) found phosphate absorption rates and the Mg-requiring, K^+-stimulated ATPase of the microsome fraction to rise in parallel. Inhibitors of the development of enhanced phosphate absorption rates also inhibited development of the ATPase. However, the amplitude of change was quite dissimilar: about 300% increase in phosphate absorption rates in 4 hr of washing *versus* about 30% increase in microsomal ATPase. Although explanations can be devised for this discrepancy, the question is raised as to whether a membrane-bound ATPase is responsible for phosphate (or other solute) transport. Is ATP the energy source for active ion transport?

This question is receiving a good deal of attention (9, 10, 15, 16, 19, 28, 37), and it seems to be widely believed that the requirement for aerobic respiration or light can be explained in terms of ATP production, at least for cation uptake. The recent work of Hodges and associates demonstrates that a Mg-requiring, cation-stimulated ATPase is present on plasmalemma from oat roots (20, 26, 27), and that the correlation between ATPase activity and ion uptake is exceptionally good (12). Lai and Thompson (23) have found that membranous vesicles from bean cotyledons have an ATPase linked to salt extrusion. Young and Sims (41) report exogenous ATP accelerates K^+ uptake by *Lemna minor*. Ratner and Jacoby (34) question the correlations between ATPase and salt uptake rates (12) on the grounds that they do not apply to organic cations.

With respect to phosphate absorption by roots, Jackson *et al.* (21) found that phosphate absorption by barley roots correlate with mitochondrial phosphorylation. Bledsoe *et al.* (4) used pretreatment with oligomycin to inhibit ATP production in corn roots, and concluded that the decline in phosphate absorption was probably due to reduction of the ATP supply to the plasmalemma. Leonard and Hodges (27) found oligomycin ineffective in inhibiting plasmalemma ATPase, thus supporting Bledsoe *et al.*

In contrast, Atkinson *et al.* (1) reported that oligomycin inhibits ATP production in carrot slices within 30 min, but salt accumulation continued undiminished for 1 hr. Atkinson and Polya (2, 32) could find no correlation between ATP levels in washed carrot and beet tissue and the rates of K^+, Na^+ and Cl^- uptake. Experimental variations were obtained with anaerobiosis, uncouplers, inhibitors of ATP formation, and adenosine-trapping agents. In washed beet root tissue with low concentrations of salt, anaerobiosis rapidly depressed salt uptake without affecting ATP concentrations significantly. Conversely, L-ethionine lowered ATP concentrations with little depression of ion uptake. Uncouplers gave inhibition of both ion uptake and ATP concentration, but the inhibition of ion uptake preceded the extensive depletion of ATP. Inhibitors of ATP production, such as oligomycin or Dio-9, had a delayed effect on ATP concentrations and ion uptake. Polya (31) found no evidence directly linking protein synthesis to ion uptake, although indirect effects were suggested. Polya and Atkinson (32) believe their results are consistent with transport mechanisms directly involving the mitochondria and oxygen-terminated electron transport.

Cram (8) studied active Cl^- influx into washed carrot root discs and concluded that uncouplers and oligomycin affected only transport into the vacuole. The Cl^- influx pump at the plasmalemma was deduced to be more closely linked to the redox reactions of the respiratory chain than to ATP production, thus supporting Atkinson and Polya.

Here we report experiments similar to those of Atkinson and Polya and with the same end in mind, but using washed corn root tissue. The correlation obtained between phosphate absorption rates and ATP concentrations is highly dependent on the treatment used to vary ATP. However, in our opinion, the results are not inconsistent with ATP being the basic energy source for active phosphate transport, provided that the mechanism has certain properties.

MATERIALS AND METHODS

Two-centimeter segments of primary root of corn seedlings (*Zea mays* L. WF9 (Tms) × M14) were taken 0.5 to 2.5 cm from the root tip and washed for 4 hr as described (24). The

[1] Research was supported by grants from the National Science Foundation (GB-37509) and the United States Atomic Energy Commission (AT-11-1-790).

[2] Dedicated to Solon A. Gordon.

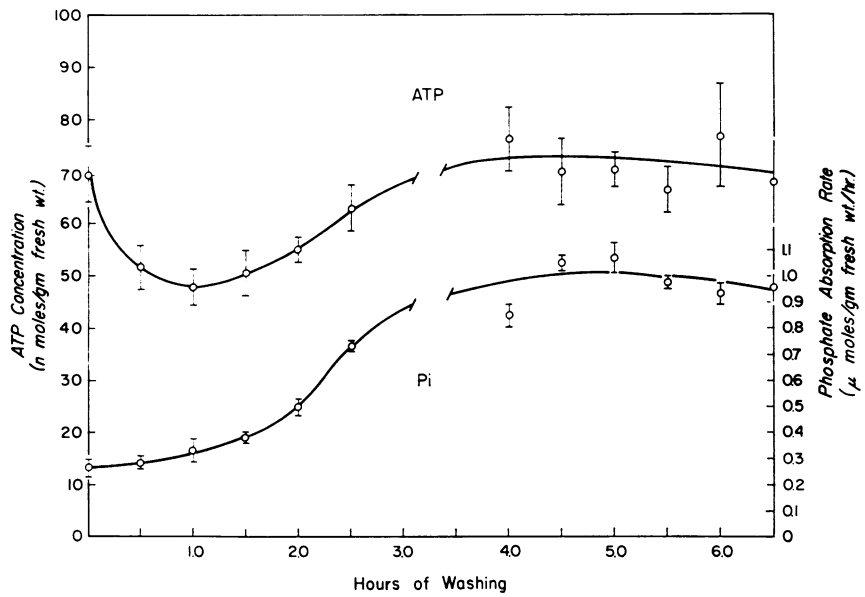

FIG. 1. Phosphate absorption rates and ATP concentration in corn root tissue. Data were collected from washing of fresh tissue and from continued washing of 4-hr washed tissues as control treatments accompanying inhibitor studies.

washing medium was 0.2 mM $CaCl_2$ plus 0.2 mM KH_2PO_4, adjusted to pH 6.0; the tissue should not have been deficient in phosphate at time of pretreatment. Pretreatment with inhibitors was in the same medium for an additional 2 hr. Phosphate absorption rates were measured, as described (24), by transferring the pretreated roots to identical solutions containing ^{32}P label for 30 min. Washing, pretreatment, and absorption solutions were held in a 30 C water bath with forced aeration. For anaerobic experiments, the pretreatment period was eliminated, with the washed roots transferred directly to the labeled absorption medium and continuously aerated with N_2 in cotton-stoppered flasks.

ATP concentrations were determined on 20-segment samples taken after the 2 hr pretreatment. The tissue was boiled for 1 min in 10 ml of 20 mM glycine buffer, pH 7.7, and immediately homogenized in an ice-jacketed Pyrex tissue grinder. (It was found that boiling for longer than 1 min caused loss of ATP, and that the grinding was essential to complete extraction. With 1 min of boiling, 5 to 15 nmoles of ATP added with the roots was completely recovered.) After centrifugation, the ATP content of the clarified extract was determined on 100 μl aliquots using an Aminco Chem-Glo photometer and 50 μl of reconstituted firefly luciferin-luciferase extract (Sigma FLE-50) as described (13).

The same extraction and assay were used in determining total adenine nucleotides except that the boiling extraction buffer was 50 mM Mg acetate plus 50 mM HEPES (Sigma) adjusted to pH 7.5. ATP + ADP was determined by incubating 0.2 ml of extract diluted with 0.2 ml of the buffer with 0.2 ml of a solution containing 40 μg of pyruvate kinase (Sigma) plus 1 μmole of trisodium phosphoenolpyruvate (CalBiochem) at 30 C for 15 min. For total AdN[3], 0.2 ml of extract, 0.2 ml of buffer, and 0.2 ml of a solution containing 40 μg of pyruvate kinase, 60 μg of adenylate kinase (Sigma), and 1 μmole of phosphoenolpyruvate were incubated for 15 min at 30 C. Calculations of energy charge were based on those of Ching and Ching (7). Comparisons with the 10-min boiling-water extraction method of Ching and Ching (7) gave up to 50% greater recovery of ATP and ADP for the method used here (*e.g.*, 66 nmoles of ATP and 50 nmoles of ADP per g of tissue *versus* 33 and 25 nmoles).

Membrane fractions were isolated by the procedures Leonard and Hodges (27) developed for oat roots. Microsomes are the fraction sedimenting from the homogenate between 13,000*g* for 30 min and 80,000*g* for 30 min. The microsomes were separated into "D-layer" and plasmalemma fractions as the interfacial layers collecting on 34 and 45% sucrose, respectively. Assays for ATPase at pH 6.0 and 9.0 were also according to Leonard and Hodges (27) except for variations in ATP, ADP, and AMP supplied (Table II).

D-MDMP was a gift from Dr. D. P. Weeks. AdN, oligomycin, L-ethionine, 2-DOG, cycloheximide, and buffers were from Sigma Chemical Co.

All experiments were confirmed by one or more repetitions.

RESULTS

ATP Concentrations during Tissue Washing. After considerable preliminary work it was decided that interpretable results could best be obtained by equilibrating the tissue with variable concentrations of inhibitors in a 2-hr pretreatment, then measuring ATP and Pi absorption rates. Although experiments were done with single inhibitor concentrations using time as a variable, the results seemed too dependent on variable rates of inhibitor uptake for useful cross comparisons. Results obtained, however, support those reported here with inhibitor concentration as a variable.

Since pretreatment involves washing, the question arises as to the effect of washing *per se* on ATP. Figure 1 shows that washing causes a decline in ATP during the induction of the enhanced absorption rates. As the Pi absorption rates reach a maximum, ATP returns to initial levels. Both RNA and protein synthesis are involved in the developmental phase (24), and the high energy demand for biosynthesis is probably responsible for the decline in ATP. Although this result might be expected, to the best of our knowledge it has not been previously observed in any induction.

It should be pointed out that we have never found any significant change in respiration rate of the tissue during the

[3] Abbreviations. AdN: adenine nucleotides; DNP: 2,4-dinitrophenol; D-MDMP: 2-(4-methyl-2,6-dinitro-anilino)-N-methylpropionamide; 2-DOG: 2-deoxyglucose.

first 2 to 3 hr of washing (24, and unpublished data) such as we might expect from a higher energy demand. The possibility of substrate limitation has not yet been investigated.

Although experiments were done with both fresh and 4-hr washed tissue, the results with washed tissue probably have the greater significance since they are not confounded with the decline in ATP during induction. The experiments which follow are with washed tissues, except as noted in Figure 10.

2,4-Dinitrophenol. Uncoupling agents are known to inhibit energy-linked ion absorption (14, 36) and to collapse the electrogenic component of cell potential (11, 17). Root tissue equilibrated with low concentrations of DNP shows greater inhibition of Pi absorption than ATP (Fig. 2). By plotting ATP *versus* Pi absorption (inset, Fig. 2) it becomes apparent that although there is a linear relationship between uncoupling of Pi transport and ATP formation, this is largely an expression of a mutual sensitivity to DNP, at least at low concentrations. Robertson (35) suggested that uncoupling could independently affect the two processes. At higher concentrations the curve breaks and extrapolates to the origin, indicating that in this range Pi absorption may be directly dependent on ATP. Indeed, it may be dependent on ATP throughout, with the dependence masked by preferential uncoupling of cell membrane potentials at low DNP concentrations. That is, if Pi transport into corn root cells is like that into corn mitochondria (13), uncoupling agents act to collapse a proton gradient which drives a Pi^--OH^- antiporter. Energy for creating the proton gradient can arise from ATP hydrolysis, either directly as a proton flux associated with hydrolysis (30), or by some modification involving intermediates such as that favored by our laboratory (13). In either case, uncoupling at cell membranes could accelerate ATP hydrolysis, lowering ATP. Slayman (37) strongly advocates proton pumping at the expense of ATP as responsible for the electrogenic component of cell potentials.

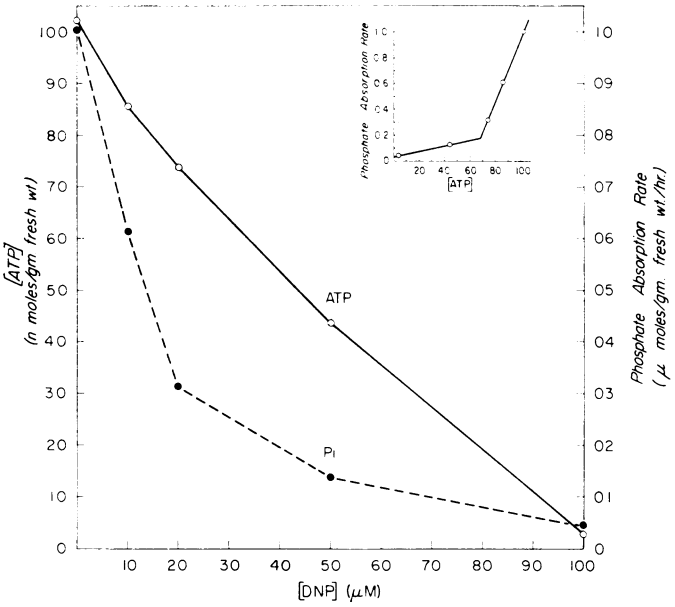

FIG. 2. Phosphate absorption rates and ATP concentration in 4-hr washed corn-root tissue pretreated for 2 hr with variable concentrations of DNP.

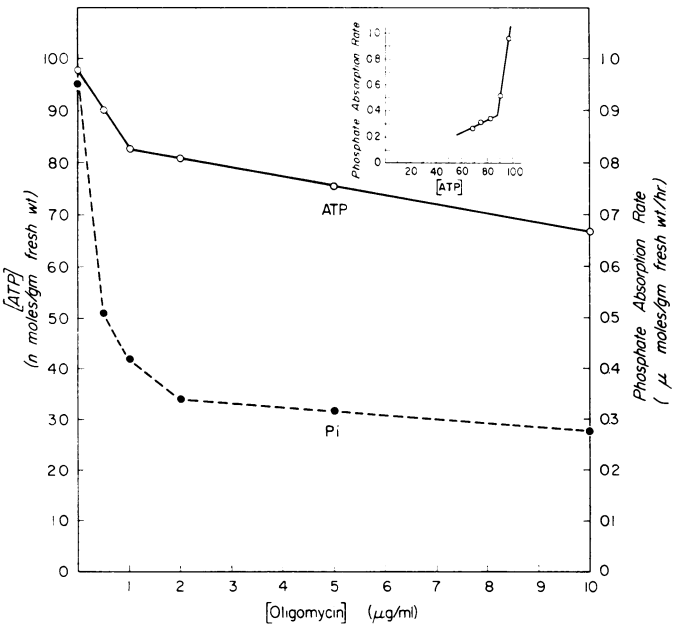

FIG. 3. Phosphate absorption rates and ATP concentration in 4-hr washed corn-root tissue pretreated for 2 hr with variable concentrations of oligomycin.

Oligomycin. Oligomycin has effects qualitatively similar to DNP, but it is quantitatively less effective in reducing ATP (Fig. 3). There appears to be an oligomycin absorption problem here. At 1 μg of oligomycin/ml, which is adequate to completely inhibit ATP production by isolated corn mitochondria (13 and unpublished data), there is a pronounced inhibition of Pi absorption with only 18% inhibition of tissue ATP concentration. Evidently, the mitochondria *in vivo* are not exposed to fully inhibitory concentrations.

However, this makes the results obtained of great interest; low amounts of oligomycin entering during the equilibration period could be preferentially absorbed at the cell surface, acting as a potent inhibitor of Pi absorption. Such a conclusion is at variance with that of Bledsoe *et al.* (4) and Jacoby and Plessner (22). Leonard and Hodges (27) find the plasmalemma ATPase of oat roots to be insensitive to oligomycin. On the other hand, oligomycin is known to inhibit both transport and the (Na^+-K^+) ATPase of animal cell membranes (40), and the uncoupler-stimulated ATPase of plant mitochondria (38). Hodges (18) originally concluded that oligomycin affected cell membrane ATPase.

When sufficient oligomycin is supplied there is a break in the ATP *versus* Pi absorption curve (inset, Fig. 3) and the line extrapolates to the origin. These results are more like those of Bledsoe *et al.* (4), who typically used oligomycin concentrations of 20 μg/ml during pretreatment.

Mersalyl. Mersalyl is a water-soluble mercurial which in low concentration will block the Pi-OH antiporter of the inner mitochondrial membrane without penetrating and inhibiting the F_1-ATPase (13, and references therein). If, as discussed above, a cell membrane ATPase is producing a proton gradient which drives Pi absorption via a similar Pi-OH transporter, it might be possible to inhibit Pi absorption with mersalyl.

Figure 4 shows that low concentrations of mersalyl do in fact act to preferentially block Pi absorption without change in ATP. At higher concentrations, ATP rises and the inhibitory effect on Pi uptake declines. This result would not be expected if sufficient mersalyl reached the mitochondria to block ATP synthesis. Consequently, the increased mersalyl must be preferentially inhibiting sulfhydryls at sites which consume ATP. At this time we have no evidence as to the nature of these sites (they might include ion-transport ATPase), but the rise in ATP is like that obtained with inhibitors of protein synthesis.

L-Ethionine. Polya and Atkinson (32) made effective use of

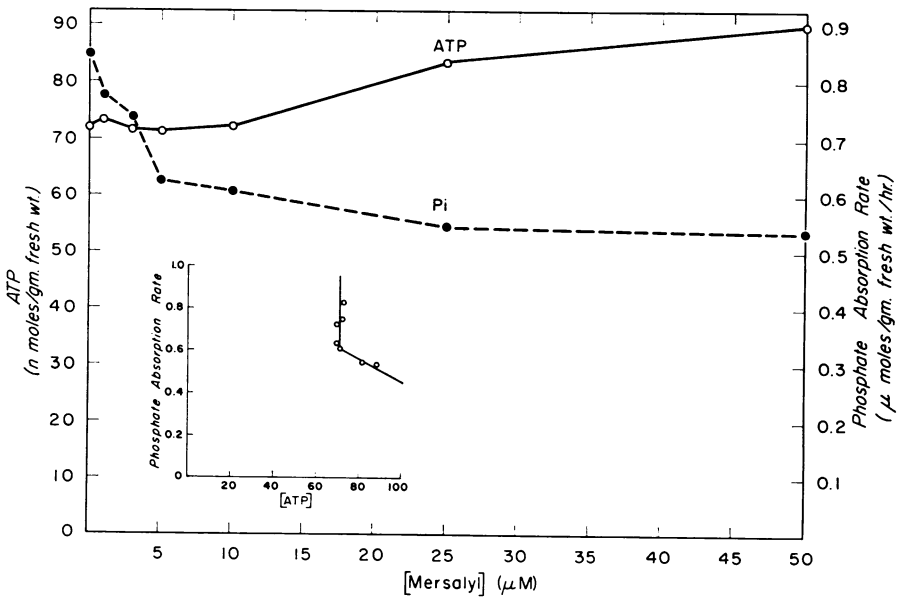

FIG. 4. Phosphate absorption rates and ATP concentration in 4-hr washed corn-root tissue pretreated for 2 hr with variable concentrations of mersalyl.

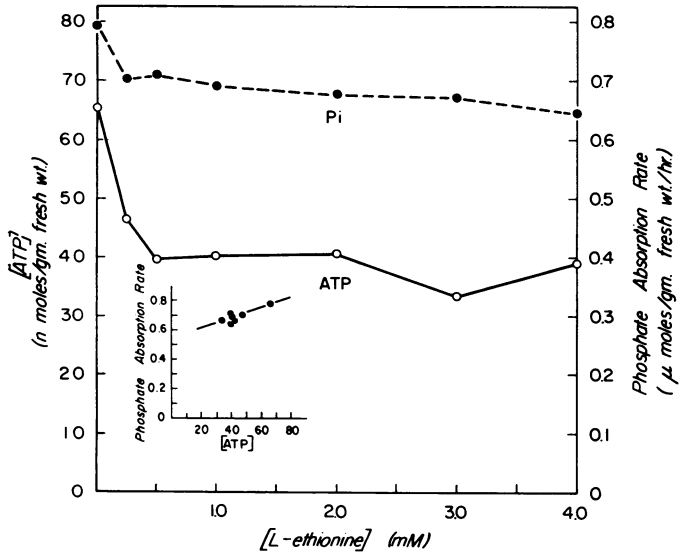

FIG. 5. Phosphate absorption rates and ATP concentration in 4-hr washed corn-root tissue pretreated for 2 hr with variable concentrations of L-ethionine.

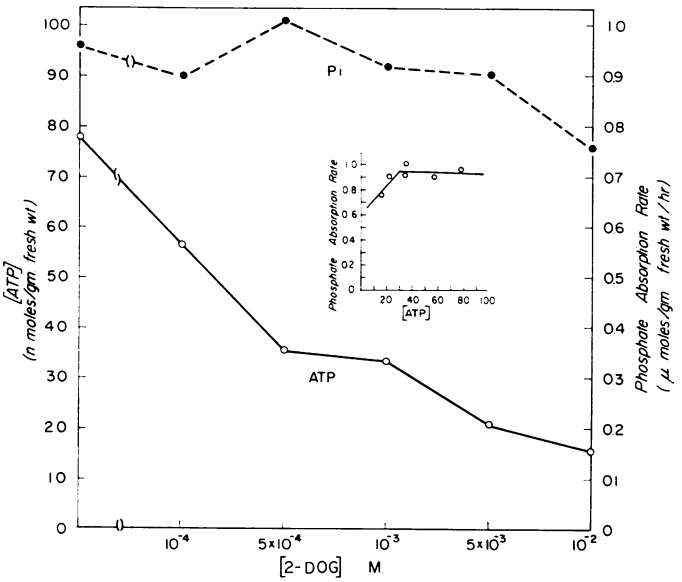

FIG. 6. Phosphate absorption rates and ATP concentration in 4-hr washed corn-root tissue pretreated for 2 hr with variable concentrations of 2-deoxyglucose.

L-ethionine to trap ATP as S-adenosylethionine, depleting the cells of adenosine. The results we obtained after 2 hr of equilibration with ethionine are reasonably similar to those they reported: there is not much decline in Pi absorption rates despite a large decline in ATP. As shown in Figure 5, the ATP *versus* Pi absorption curve does not extrapolate to the origin. However, ethionine is at best only about 50% effective in lowering ATP, and it might be argued that greater depletion is needed before the membrane ATPase is affected.

2-Deoxyglucose. 2-DOG has been used as phosphate trap in cells since it reacts with ATP in the presence of hexokinase to produce a nonmetabolizable 2-deoxyglucose 6-phosphate. In prolonged incubations, it also induces degradation of AdN via deamination of AMP to inosine monophosphate followed by dephosphorylation to inosine (29). 2-DOG was found to be much more effective in lowering ATP levels than was ethionine (Fig. 6). Only at the higher concentrations of 2-DOG was there indication of lowering Pi absorption rates. With 2-DOG, the lowering of cellular Pi might accelerate Pi absorption.

Anaerobiosis. Due to technical difficulties, experiments with nitrogen gassing were performed with time as a variable, rather than equilibration with concentrations (Fig. 7). Unlike Polya and Atkinson (32), we found ATP and Pi absorption rates to decline in parallel for the first 1 to 1.5 hr. The early portion of the ATP *versus* Pi absorption-rate curve is linear and extrapolates to the origin (inset, Fig. 7). Thereafter, there is a sharp break in the curve with ATP only slightly declining while Pi absorption drops rapidly, behaving similarly to beet storage-root slices (32). We investigated whether anaerobic conditions might be destroying membrane semipermeability, but could find no evidence for this in terms of K^+ or nucleotide

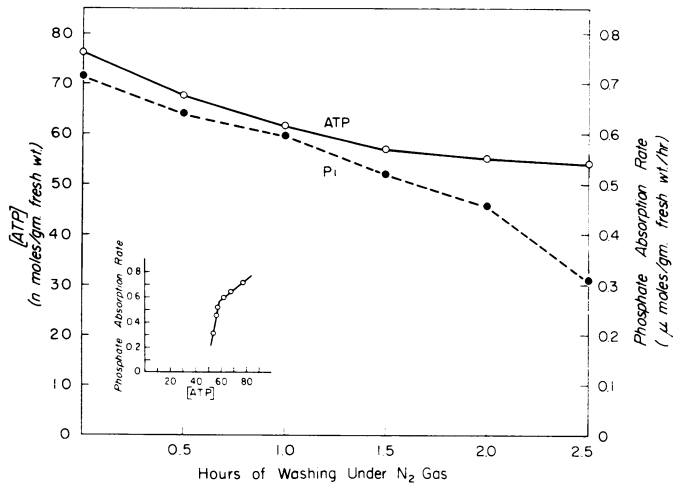

FIG. 7. Phosphate absorption rates and ATP concentration in 4-hr washed corn-root tissue at various periods under N_2 gassing.

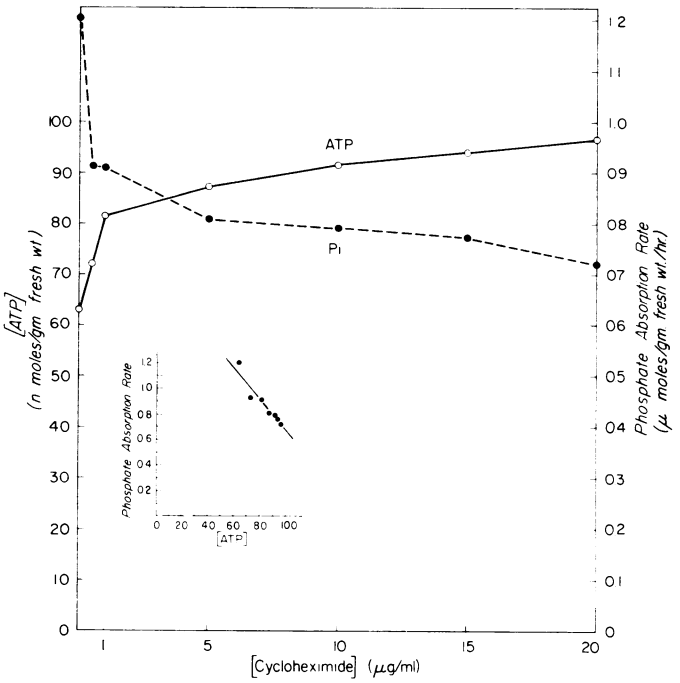

FIG. 8. Phosphate absorption rates and ATP concentration in 4-hr washed corn-root tissue pretreated for 2 hr with variable concentrations of cycloheximide.

leakage from the roots. This does not preclude anaerobic conditions indirectly affecting membrane transport enzymes.

Inhibition of Protein Synthesis. Two inhibitors of protein synthesis were used, cycloheximide and D-MDMP; D-MDMP inhibits peptide chain initiation (39). Since the tissue was prewashed for 4 hr, the inhibitors were applied after the development of the enhanced Pi absorption capacity (Fig. 1).

Both inhibitors acted to increase ATP, probably by blocking the energy demand for protein synthesis (Figs. 8 and 9). At the same time that ATP was rising, Pi absorption rates declined, although with somewhat different concentration responses for the two inhibitors. There are unresolved problems with cycloheximide acting to partially inhibit Pi absorption (24), which may account for the rapid initial drop in Pi absorption rates in Figure 8. The ATP *versus* Pi absorption-rate curves for both inhibitors are linear, but with negative slopes. It is probable that among the proteins turning over in the tissue are enzymes associated directly or indirectly with Pi transport. Blocking protein synthesis has two consequences which thus correlate: a rise in ATP and a decline in Pi absorption rates.

Energy Charge. Reduction of ATP with ethionine or 2-DOG is inherently different from uncoupling or blocking ATP formation with DNP or oligomycin in that levels of adenylates or phosphate are being depressed with no assurance that the proportion of ATP is lowered. Experiments were done where energy charge (ATP+0.5ADP/ATP+ADP+AMP) was measured after 2 hr of pretreatment in L-ethionine or 2-DOG. The results are given in Table I.

Both ethionine and 2-DOG reduced the total AdN content. The concentration of total AdN in control corn-root tissue is slightly higher and the proportions different than the ATP/ADP/AMP (10:3:1) described by Bielski (3) as typical, thus giving a low energy charge of about 0.6. Chapman *et al.* (5) note that plant tissues tend to have lower energy charge than do microorganisms and animal tissues (7).

Two hr of pretreatment in 1 mM ethionine or 2-DOG dropped the energy charge by about 25% (Table I) with little effect on Pi absorption rates (Figs. 5 and 6). However, there is

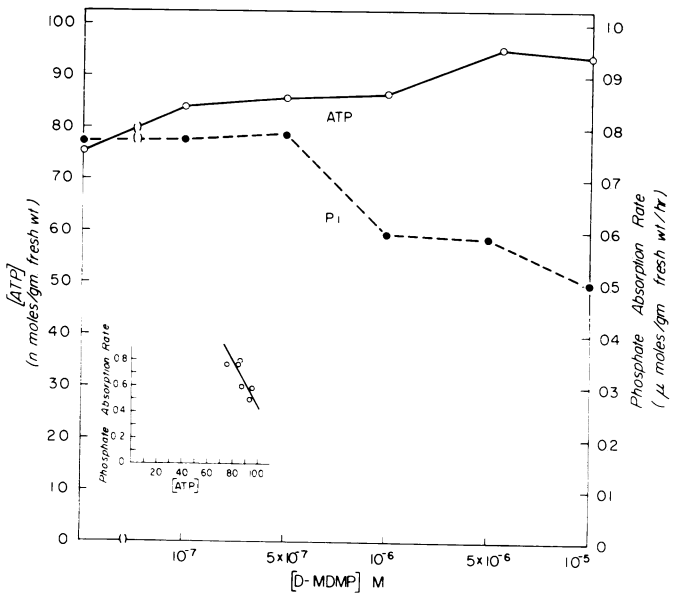

FIG. 9. Phosphate absorption rates and ATP concentration in 4-hr washed corn-root tissue pretreated for 2 hr with variable concentrations of D-MDMP.

Table I. *Energy Charge in Corn Root Tissue as Influenced by 4 Hr Washing and by Treatment with* L-*Ethionine or 2-DOG*

	ATP	ADP	AMP	ΣAdN	Energy Charge
	nmoles/g fresh wt				
Fresh tissue	64	61	28	153	0.62
Washed tissue (4 hr) + 2 hr treatment	71	52	38	161	0.60
Control	67	53	34	154	0.61
L-Ethionine (1 mM)	36	31	45	112	0.46
L-Ethionine (3 mM)	30	30	40	100	0.45
2-DOG (1 mM)	35	45	48	128	0.45
2-DOG (10 mM)	20	29	42	91	0.38

Table II. *Effect of ATP Concentration and Energy Charge on the K⁺-stimulated ATPase of Microsomes, Plasmalemma, and D-layer Obtained from Homogenates of Corn Roots*

Adenine Nucleotides	ATPase			
	pH 6.0		pH 9.0	
	Microsomes	Plasmalemma	Microsomes	D-layer
	μmoles Pi/mg protein·hr			
Energy charge[1]				
0.6	11.3	9.1	3.7	5.2
0.4	10.7	8.5	3.3	4.0
0.2	6.9	5.9	2.1	2.1
ATP (mM)[2]				
3.00	16.4	13.7	5.8	6.5
2.04	13.0	11.3	7.1	5.0
1.53	10.8	10.3	3.1	4.3
0.87	7.6	10.3	2.4	3.6

[1] Adenine nucleotide mixtures were (mM): 0.6: 2.04 ATP, 1.65 ADP, 1.08 AMP; 0.4: 1.53 ATP, 0.78 ADP, 2.49 AMP; 0.2: 0.87 ATP, 0.15 ADP, 3.78 AMP. Incubation was for 20 min at 38 C in 1 ml volumes of 1.5 mM $MgSO_4$, 50 mM KCl, 33 mM tris-MES buffer with 10 to 15 μg of protein.

[2] No ADP or AMP provided.

no way of judging the significance of this unless it is known how energy charge affects the membrane-bound ATPases.

Microsomes, plasmalemma, and D-fraction were isolated from corn root homogenates by the procedures of Leonard and Hodges (27). ATP was provided for the K⁺-stimulated ATPase assays in arbitrary proportions of 4.8 mM total AdN such that the energy charge would be 0.6, 0.4, and 0.2. In addition, ATP alone, in the same concentrations as used for energy charge mixture, was also used in ATPase determination. The results are given in Table II.

Reducing initial energy charge from 0.6 to 0.4—which covers the range found (Table I)—gives about a 7% reduction in ATPase activity of the plasmalemma. Using ATP only, the reduction in activity between 3.0 and 1.5 mM was 25%, slightly greater than that found by Leonard and Hodges (27) for oat-root plasmalemma. Assuming that AdN are confined to the cytoplasm, and that the cytoplasmic layer provides 0.03 to 0.05 ml/g tissue, concentrations of total AdN would be about 3 to 5 mM.

Fresh Tissue. Figure 10 is a summary for fresh tissue of the same experiments performed with washed tissue (insets, Figs. 2 to 7) except that cycloheximide was not used. The results are qualitatively the same, but we consider them to have less quantitative significance because the inhibitors must also affect those processes involved in induction and development of enhanced solute uptake rates.

DISCUSSION

It is hazardous to extrapolate correlations such as these to firm conclusions about biochemical events at membranes. However, the data can be examined for consistency with the hypothesis that ATP is the energy source for ion transport.

Unlike Polya and Atkinson (32), we conclude that the data are not inconsistent with ATP as the energy source. The failure of L-ethionine or 2-DOG to cause marked depression of Pi uptake might be explained in terms of inadequate depression of energy charge, a matter which needs special and intensive study. We find N_2 gassing to produce parallel decreases in ATP and Pi absorption for the 1st hr. The disproportionate inhibition of Pi absorption thereafter can be as logically attributed to build-up of an inhibitory product (perhaps as simple as bicarbonate, acetaldehyde, or ethanol) as to a requirement for electron transport to oxygen directly connected to ion transport.

For consistency, however, the corn root-cell membrane ATPase (plasmalemma or tonoplast or both) would have to have certain properties, not all of which are manifested in *in*

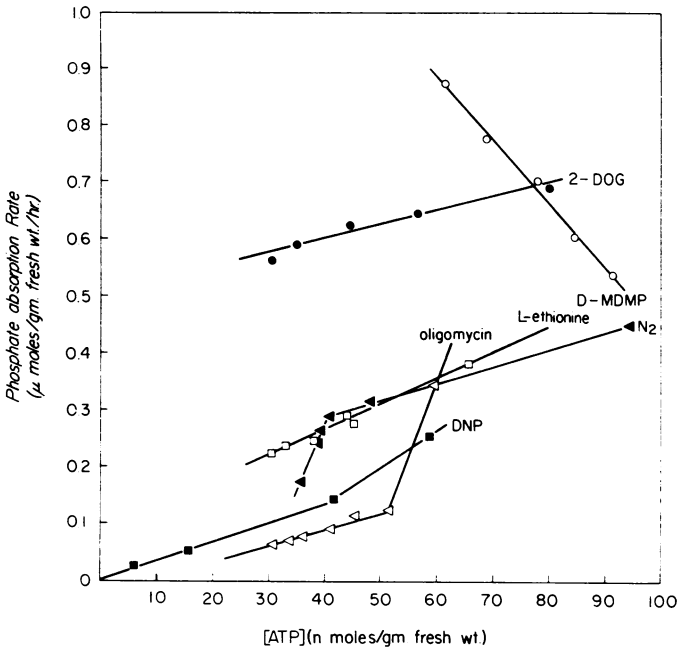

FIG. 10. Summary of correlations between phosphate absorption rates and ATP concentrations for fresh root tissue pretreated for 2 hr in concentrations of inhibitors. Experiments were performed as in Figures 2 to 9.

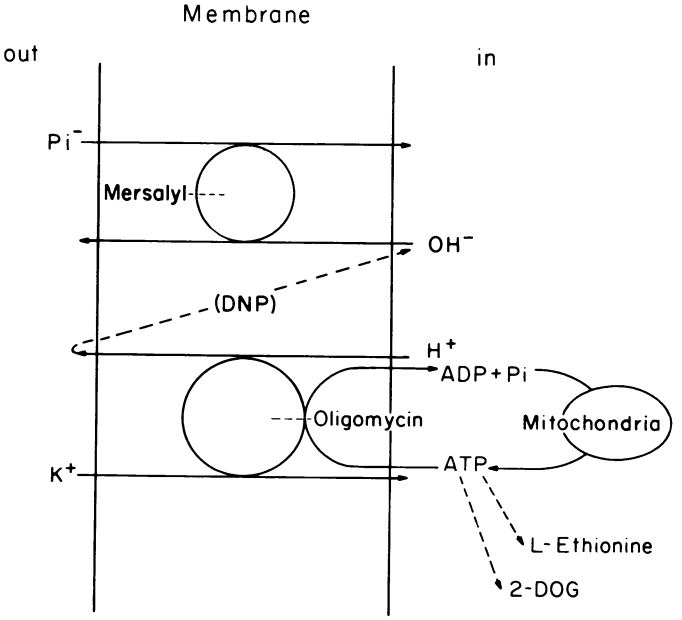

FIG. 11. Schematic representation of the apparent requirements for a membrane ATPase transporting phosphate.

vitro ATPase studies. (*a*). Membrane-bound ATPase should be inhibited by low concentrations of oligomycin. (*b*). The ATPase should also be sensitive to uncouplers, which signifies creation of a proton gradient at the expense of ATP hydrolysis. (*c*). The proton gradient created should drive Pi transport via a mersalyl-sensitive exchange transporter similar to that of the mitochondrial inner membrane. (*d*). The ATPase should operate with reasonable efficiency at energy charge as low as 0.4. (*e*). The ATPase (or the Pi transporter) should have a half-life of a few hours, with constant protein synthesis needed for maintenance. (*f*). Since K^+ is also being absorbed from the potassium phosphate solution (24), the ATPase must also energize K^+ transport.

The simplest type of ATPase mechanism consistent with these properties is schematically illustrated in Figure 11. ATP hydrolysis is somehow linked to H^+-K^+ exchange, probably not stoichiometrically neutral if the process is electrogenic (*i.e.*, proportionately greater exit of H^+ would be required for creation of an energy-linked membrane potential, negative inside). The proton gradient thus created could drive the Pi-OH transporter, much as it is believed to occur in mitochondria (6, 13). Uncouplers would collapse the gradient, and oligomycin, or N,N′-dicyclohexylcarbodiimide (27), would block the ATPase. So assembled, the observations come very close to ATPase energized transport as visualized by Hodges (19), and owe much to Mitchell's concept of proton-pumping ATPase (30).

The above properties are those needed for consistency between the observations made and the assumption that ATPase provides the energy for potassium phosphate transport. It is disturbing that membrane-associated ATPases are not sensitive to oligomycin (27) or uncouplers (T. K. Hodges, personal communication); but with one exception (23) neither are they reported to transport salt. There is precedent for the loss of oligomycin sensitivity in the studies of mitochondrial ATPase (33). Resolution of the problem will have to come with more intensive work with *in vitro* membrane systems, particularly with ATPase systems that can transport salt.

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