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Induction and Development of Increased Ion Absorption in Corn Root Tissue[1]

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ABSTRACT

Washing excised or intact primary roots of corn (*Zea mays* L., WF9 × M14) in aerated distilled water or dilute salt solutions for 2 hours induced doubling of the rate of accumulation of various nutrient ions and solutes. This response to washing depended upon aerobic metabolism, but involved no increase in aerobic respiration. Excision of root tissue was not required as the effect could be obtained with intact root systems. Increased phosphate absorption followed afer a lag period of 30 to 40 minutes and continued for 6 hours before leveling off at about 3.5 times the initial rate. Chloramphenicol was not inhibitory to the development of increased absorption, while inhibitors of RNA and protein synthesis were. Auxins and kinetin were also inhibitory, but so was the antiauxin, 2,4,6-trichlorophenoxyacetic acid.

Kinetic analyses of phosphate and potassium (^{86}Rb) absorption showed no change in apparent Michaelis constant. Electron microscopic examination and analysis for membrane protein and lipid-phosphate content after washing showed no proliferation of membrane or other changes in cell structure and composition. It appears that washing augments or activates existing membrane transport mechanisms by processes involving protein synthesis.

There are several reports on the enhanced ion accumulation rates which can be obtained by washing of vegetative stem and root tissue. Unlike the situation with washed slices of dormant storage tissue (11, 12) there is little or no augmentation of respiration. Thus the changes leading to increased ion transport rates are not confounded with those giving an increased respiratory energy supply, and it should be possible to identify more closely the alteration in transport mechanisms.

Palmer and Loughman (20) found that washing pea epicotyl tissue substantially increased phosphate absorption and esterification. There was a slight decline in respiration rate and no change in the sensitivity of respiration to respiratory inhibitors. Increased phosphate absorption could not be attributed to loss of phosphate during washing. Prior to washing, phosphate absorption was not very sensitive to respiratory inhibitors, while after washing phosphate absorption was closely correlated with respiratory metabolism. Oat, wheat, and barley tissue also responded to washing, while cotton and sunflower did not.

Bieleski (1) suggested that low affinity transport mechanisms dominate in fresh tissues of celery and cabbage. Washed tissue develops additional, high affinity mechanisms. Hancock (7, 8) also reached this conclusion for 3-O-methyl glucose absorption in squash hypocotyl segments. He obtained evidence by use of cycloheximide and actinomycin D that RNA and protein synthesis were implicated in the washing response. Calcium ion was not (8). *Hypomyces* infection induced changes in adjacent healthy tissue similar to those induced by washing.

Rains (24) showed that washing bean segments induced a high affinity potassium-absorbing mechanism; concurrently sodium absorption rates declined. The induction was temperature dependent and sensitive to metabolic inhibitors and could not be explained by changes in efflux rates of either K^+ or Na^+. More recently, Rains and Floyd (25) reported that Ca^{2+} promoted the washing response and gave an increase in respiration rate. Cycloheximide inhibited the washing response.

Sacher (26) observed an increase in the rate of orotic acid, glucose, and phenylalanine uptake by bean endocarp tissue pretreated in water for 18 to 25 hr. A synthetic auxin, α-naphthalene acetic acid, largely prevented the increase. Auxin inhibition of the washing response has also been observed with 2,4-D by Palmer and Blackman (19). However, 2,4,6-T,[3] which has little auxin activity, was also inhibitory to the enhancement of salt absorption during washing (18).

Macklon and Higinbotham (16) measured cell electropotentials in washed pea epicotyl segments and found a substantial rise which preceded the increase in rate of K^+ or NO_3^- absorption.

Root tissue also responds to washing with enhanced salt uptake rates. Laties and Budd (13) showed that washing segments of corn roots or stelar and cortical tissue from corn roots for 24 hr increased Cl^- uptake rate. For steles, the increase was up to 20-fold. Lüttge and Laties (15) reported 10-fold increases in K^+ absorption rates for washed corn root steles by a process sensitive to actinomycin D, puromycin, and low temperatures. Kinetic analysis showed no increase in absorption affinity.

Yu and Kramer (29, 30) were unable to confirm this washing response in either cortical or stelar tissue of corn. However, Hall *et al.* (5) were able to show that washing for 24 hr induced a 10-fold increase in chloride uptake by stele and cortex of corn roots grown under nonsterile conditions. Under sterile conditions the increase was only 2- to 3-fold; hence, they concluded that bacteria can contribute to the uptake.

Pitman (21) aged excised barley roots for 4 hr in dilute

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[3] Abbreviation: 2,4,6-T: 2,4,6-trichlorophenoxyacetic acid.

$CaSO_4$ and found an increased net K^+ uptake at the expense of Na^+ uptake compared to fresh roots. The difference was attributed to K^+ efflux during washing. No differences in tracer uptake were observed.

More recently, Pitman *et al.* (22) reported on cell potentials of barley root cortical cells during aging. There was a rapid and dramatic rise in negative potential which leveled out after about 6 hr.

A number of years ago it was observed in our laboratory in connection with other work that holding segments of corn or soybean root tissue in aerated water for as little as 2 hr would double the rate of ^{32}P or ^{86}Rb absorption. There was no change in respiration rate. We here report an investigation of this phenomenon for corn root segments. In general, the results support the observations made in Laties' laboratory. Washing does induce an enhanced rate of solute absorption in corn roots grown under highly aerobic conditions, but there is no change in apparent *Km*. Development of the mechanism requires aerobic respiration and is sensitive to inhibitors of RNA and protein synthesis. The induction is due to submersion of the roots, not to excision, and produces absorption rates comparable to those of solution-grown roots.

MATERIALS AND METHODS

Plant Material. Corn seedlings (*Zea mays* L., WF9 × M14) were obtained by placing seeds embryo down in glass trays which contained several layers of paper towels saturated with 0.1 mM $CaCl_2$. The trays were covered with plastic food wrap, perforated to allow for air exchange, and placed for 3 days in the dark at 29 C and high humidity. Additional 0.1 mM $CaCl_2$ was added after 2 days to maintain saturation of the paper towels. Root segments from seedlings grown in this manner are termed "tray-grown roots" and were used throughout this study unless otherwise specified.

Corn seedlings were also obtained by placing imbibed (12 hr) seeds between two layers of cheesecloth stretched over a 2-liter polyethylene container. The containers were either partially or completely filled with 0.1 mM $CaCl_2$ and transferred to the growth room described above; vigorous aeration was provided. Root segments from 3-day-etiolated seedlings grown in partially filled containers where the roots did not extend into the solution are termed "vapor-grown roots," while those from seedlings grown in completely filled containers where the roots were completely immersed in solution are called "solution-grown roots."

Unless otherwise noted the 0.5- to 2.5-cm section behind the tip of the primary root of 3-day-etiolated seedlings was used in these experiments. Batches of 20 segments (about 300 mg fresh weight) were tied in bags of cheesecloth leaving a 20- to 30-cm length of string to facilitate movement from one solution to the next. This procedure is described in detail by Epstein *et al.* (4).

Washing Procedure. The tissue was washed in well aerated 0.2 mM $CaCl_2$ solution at 30 C or ice temperatures for various periods of time with the additives indicated in the text, 100 ml of solution per 20 segments. The pH of this solution ranged from 5.5 to 5.8. When other substances were added to the washing solution, the pH was adjusted if needed to this range with 0.01 N $Ca(OH)_2$. As noted later, the washing response could be obtained equally as well in glass-distilled water, but calcium was routinely used (3).

Absorption Measurements. Root sections were incubated in 0.2 mM $CaCl_2$-0.2 mM phosphate (K^+ salt, pH 6.0) labeled with ^{32}P for 1 hr at 30 C with aeration. The tissue was rinsed for 30 sec with ice-cold water and then placed in unlabeled uptake solution for 30 min exchange at ice temperatures. Where the uptake of other labeled solutes was tested (*e.g.*, Table II) these were added at 0.1 mM concentration to the $CaCl_2$-potassium phosphate solution, except for ^{86}Rb and ^{36}Cl where 0.2 mM KCl was substituted for the potassium phosphate. Blotted and weighed sections were counted in a Packard scintillation counter. A thixotropic gel was added to the scintillation fluid to keep the roots uniformly suspended.

Potassium content of the root sections was determined by emission flame photometry on nitric acid digests with Li^+ as an internal standard.

Tissue Analysis. Phospholipid extraction and analysis was done by the method of Hall and Hodges (6). Protein was determined according to the method of Lowry *et al.* (14).

RESULTS

Characterization of the Washing Response. In line with previous investigations, temperature and aeration proved critical to development of enhanced salt absorption rates (Table I). Although the physical act of washing may be the inducer of the response, the development of increased rates of absorption is dependent on aerobic metabolism. As with pea epicotyl (20), but not with bean stem (25) and corn stele (5), there was no increase in respiration rate. This result has been repeatedly confirmed; it is of major importance, since the increased phosphate absorption cannot be attributed to increased respiratory metabolism.

Various washing media were investigated. Equal enhancement of phosphate absorption rates was secured by washing in double distilled water, 0.2 mM $CaCl_2$, 0.2 mM KCl, 0.2 mM potassium phosphate (pH 5.8 and 7.0) plus 0.2 mM $CaCl_2$, and 0.2 mM sodium phosphate (pH 5.8 and 7.0) plus 0.2 mM $CaCl_2$.

A limited survey was made to determine how general the enhancement of absorption rates was (Table II). Of those solutes tested, only calcium and acetate showed no response. Whatever facet of the transport mechanism is developed by washing, it is one which has broad application in solute absorption.

Root tissue from other species was checked for the washing response. Three hours of washing in 0.2 mM $CaCl_2$ increased phosphate absorption rates of the 0.5- to 2.5-cm segment of the root as follows (percentage of fresh tissue): corn, 280; barley, 150; oat, 200; soybean, 250; pea, 140; cucumber, 170.

Table I. *Respiration Rates and Phosphate Absorption Rates as Affected by Washing*

Respiration rate was determined by standard manometric techniques. The Warburg flask contained: 0.2 ml of 20% KOH in the center well along with a filter paper wick; 3 ml of 0.2 mM $CaCl_2$ plus 0.2 mM KH_2PO_4-K_2HPO_4, pH 6.0, in the main compartment; and 20 root segments. The bath temperature was 30 C. Oxygen consumption was linear during the 2-hr recording period.

Washing Pretreatment	Respiration	Phosphate Absorption
	μl O_2/g fresh wt·hr	*μmole/g fresh wt·hr*
None (fresh tissue)	620	0.22
2 hr, 2 C	612	0.18
2 hr, 30 C	608	0.47
None (fresh tissue)		0.20
2 hr, 2 C, aerated		0.16
2 hr, 30 C, aerated		0.49
2 hr, 30 C, N_2		0.15
2 hr, 30 C, O_2		0.50
2 hr, 30 C, no aeration		0.27

Table II. *Accumulation of Various Solutes by Corn Root Segments before and after 2 hr of Washing*

All uptake solutions contained 0.2 mM $CaCl_2$ and 0.2 mM potassium phosphate, pH 6.0, except for ^{86}Rb and ^{36}Cl, which contained 0.2 mM KCl and 0.2 mM $CaCl_2$. The uptake solutions for labeled organic solutes contained 0.1 mM appropriate carrier.

Solute	Absorption		Washed/Fresh
	Fresh control	Washed	
	μmoles/g fresh wt·hr		
Phosphate (^{32}P)	0.22	0.49	2.2
Potassium (^{86}Rb)	1.18	2.04	1.7
Chloride (^{36}Cl)	0.39	0.81	2.1
Glycine (^{14}C)	0.18	0.36	2.0
Glucose (^{14}C)	0.29	0.42	1.5
GMP (^{14}C)	0.016	0.028	1.8
Calcium (^{45}Ca)	0.41	0.38	0.9
Acetate (^{14}C)	0.12	0.11	0.9

Table III. *Effect of Washing on the Net Accumulation of Potassium by Corn Root Tissue*

K^+ content was determined by flame photometry on acid digests of the root tissue before and after an uptake period of 1 hr in 0.2 mM KH_2PO_4, pH 6.0, + 0.2 mM $CaCl_2$.

Washing Pretreatment	K^+ Content		K^+ Accumulation
	Initial	Final	
	μmoles/g fresh wt		*μmoles/g fresh wt·hr*
None	44.4 ± 1.4	41.5 ± 0.8	−2.9 ± 0.6
2 hr, 2 C	40.2 ± 0.7	39.2 ± 0.6	−1.0 ± 1.3
2 hr, 30 C	40.5 ± 0.8	44.2 ± 1.9	+3.7 ± 1.0

The question arose as to whether washing enhanced net salt accumulation or simply accelerated ion exchange. Potassium contents were followed, since K^+ exists only in the ionized form and is known to leach readily (17). Net K^+ accumulation was found in tissue washed at 30 C, but not in freshly excised tissue or that washed at 2 C (Table III). It was observed in these experiments that during the inductive washing considerable K^+ leaked from tissue (in both cold and warm treatments). Similar observations have been made before (17). Since K^+ is also lost at low temperatures, the enhancement of absorption rates cannot be attributed to development of low salt tissue.

Primary roots were cut into serial segments to determine if the washing response was a function of cell age. Figure 1 shows that it is not. Mature tissue responded almost as readily as growing tissue. However, in preliminary work with soybean and oat root tissue (unpublished data) the apical 0.5 cm was found not to show promotion of ^{86}Rb uptake upon washing.

The time course of the development of enhanced phosphate absorption is shown in Figure 2. There is approximately a 30-min inductive lag period, followed by about 4 hr of development of enhanced capacity for transport. After about 8 hr there is some decline, possibly due to senescence of the tissue. The shape of the curve indicates that bacteria cannot be contributing significantly to the phosphate uptake, since otherwise it would rise throughout the course of washing. In substantiation of this, inclusion of 50 μg/ml chloramphenicol was without effect on ion uptake or the washing response (see below).

Inasmuch as washing in a phosphate buffer also enhanced ion absorption, one would expect the time course of phosphate uptake by fresh tissue to show a rising absorption curve during the initial hours. This proved to be the case (Fig. 3). Again, there is a lag period before the rate increases.

Kinetic analyses of phosphate (^{32}P) and potassium (^{86}Rb) absorption rates for fresh and washed tissue are summarized in Table IV. The apparent *Km* values do not change significantly with washing. This result is like that of Lüttge and Laties (15) for corn roots, but is unlike that obtained with some other vegetative tissues (7, 8, 20, 24, 25). In corn roots the enhancement of accumulation rate must lie not with the formation of different and more efficient transport agents (or carriers), but rather with a greater quantity or a greater turnover of existing agents.

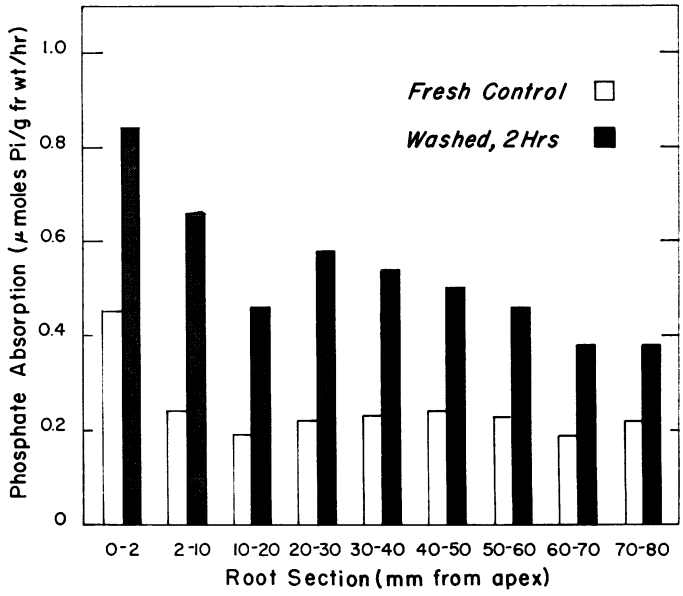

FIG. 1. Effect of cell maturation on the response to washing of corn root tissue.

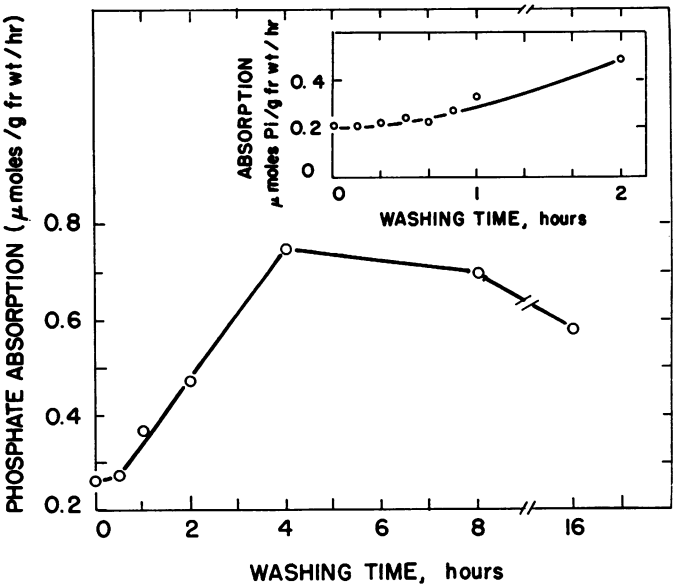

FIG. 2. Time course for the induction of an increase in the rate of phosphate absorption by corn root tissue. Root tissue washed for the indicated periods was removed and assayed for capacity to absorb phosphate over 1 hr.

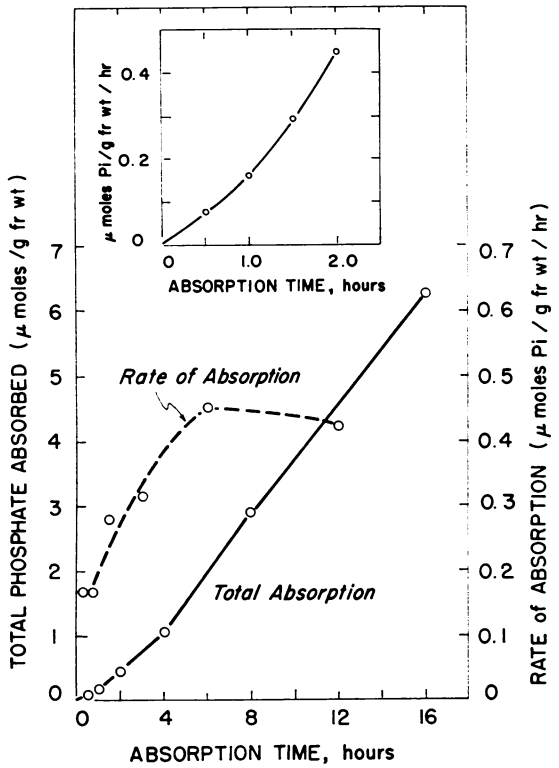

FIG. 3. The absorption of phosphate by corn root segments with time. The rate of absorption (dashed line) was calculated by subtracting the difference in total absorption between two adjacent points on the curve and dividing by the difference in time of the corresponding points.

Table IV. *Kinetic Constants for Phosphate and Potassium (^{86}Rb) Absorption by Fresh and 8-hr-washed Corn Root Segments*

Kinetic constants were calculated by linear regression analysis. The absorption technique was the same as that described in "Materials and Methods," except that the segments were not bound in cheesecloth bags during absorption, and the uptake period was 30 min.

Solute	*Km*		V_{max}	
	Fresh	Washed	Fresh	Washed
	mM		*μmoles/g fresh wt·hr*	
Phosphate (^{32}P), 0.05–0.25 mM range	0.020	0.026	0.24	0.78
Potassium (^{86}Rb), 0.05–0.25 mM range	0.016	0.015	1.20	3.79

Inhibitor Studies. Phosphate absorption was equally sensitive to respiratory inhibitors in fresh and washed tissue (Table V). Hence, the development of increased absorption rates does not involve any detectable change in dependence on respiratory metabolism.

Table VI shows the effect of hormones and cyclic AMP during washing on subsequent phosphate absorption. Gibberellic acid and cyclic AMP had little effect, but kinetin, IAA, and 2,4-D did give clear inhibition as noted in earlier reports (8, 18, 19, 24, 26). The greatest inhibition, however, was secured with the antiauxin, 2,4,6-T. We checked the auxin properties of the 2,4,6-T in cucumber radicle growth inhibition: at 10 μM the 2,4,6-T gave 11% inhibition while the same concentration of 2,4-D gave 64% inhibition. It seems probable that the auxin inhibition of the development of increased salt absorption rates may not reside in the auxin properties of the molecule.

Inhibitors of protein and RNA synthesis were examined for their effect on the washing response (Table VII) and on phosphate absorption (Fig. 4). Chloramphenicol at bacteriostatic concentrations has no effect on the washing response or on uptake. Actinomycin D, cycloheximide, and 6-methylpurine strongly inhibited the development of increased absorption rates, which is in agreement with previous investigations (7, 15, 25), and which supports the general supposition that RNA

Table V. *Effect of Aeration, Temperature, and Metabolic Inhibitors on Phosphate Absorption by Fresh and 2-hr-washed Corn Root Tissue*

Inhibitors were present only during the absorption period (1 hr).

Treatment	Rate of Phosphate Absorption	
	Fresh control	Washed 2 hr
	% inhibition	
Anaerobiosis (N_2 gas)	88.1	86.8
Low temperature (0–2 C)	99.0	99.1
Potassium cyanide, 1 mM	66.7	57.1
Sodium azide, 1 mM	63.2	64.8
Dinitrophenol, 0.1 mM	70.8	69.4

Table VI. *Effect of Hormones and Other Substances during Washing on Subsequent Phosphate Absorption*

Washing Pretreatment	Phosphate Absorption[1]
	% of washed control stimulation
2 hr (washed control)	100.0
2 hr + 0.1 mM gibberellic acid	96.0
2 hr + 5 μM kinetin	55.9
2 hr + 0.1 mM IAA	41.0
2 hr + 0.1 mM 2,4-D	52.4
2 hr + 0.1 mM 2,4,6-T	−14.9
2 hr + 0.2 mM 3′5′ cyclic AMP	82.0
2 hr + 0.2 mM dibutyryl 3′5′ cyclic AMP	91.5

[1] The stimulation in the rate of phosphate absorption due to 2 hr of washing at 30 C.

Table VII. *Effect of Chloramphenicol and Inhibitors of RNA and Protein Synthesis on Washing of Corn Root Tissue*

Washing Pretreatment	Phosphate Absorption[1]
	% of washed control stimulation
2 hr (washed control)	100.0
2 hr + 25 μg/ml chloramphenicol	94.7
2 hr + 50 μg/ml chloramphenicol	100.0
2 hr + 20 μg/ml actinomycin D	11.5
2 hr + 10 μg/ml cycloheximide	−29.2
2 hr + 0.5 mM 6-methylpurine	13.2
2 hr + 2.5 mM 5-fluorouracil	96.3

[1] The stimulation in the rate of phosphate absorption due to 2 hr of washing at 30 C. The inhibitors were not present during the phosphate absorption period.

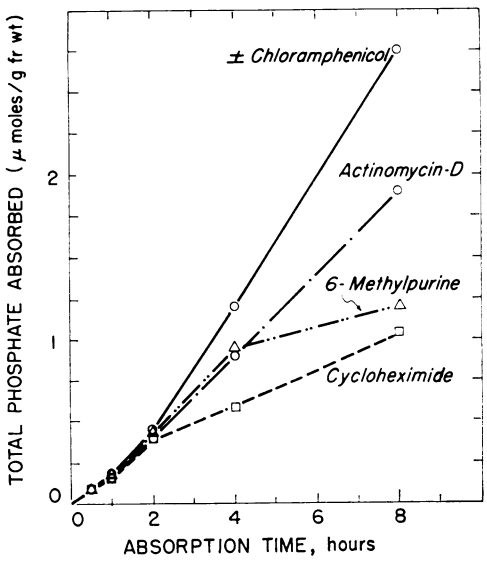

FIG. 4. The effect of chloramphenicol and inhibitors of RNA synthesis and protein synthesis on phosphate absorption by freshly cut corn root tissue. Chloramphenicol, 50 μg/ml; actinomycin D, 15 μg/ml; 6-methylpurine, 0.5 mM; and cycloheximide, 10 μg/ml.

and protein synthesis underlie the development of the washing response. None of these three inhibitors affects phosphate accumulation over the first 2 hr when added directly to the uptake medium, but thereafter they were effective (Fig. 4). Cycloheximide has been reported to inhibit ion transport (2).

The fact that 5-fluorouracil did not inhibit the washing response (Table VII) indicates that ribosomal and soluble RNA synthesis is not critical to the response (10). Presumably, messenger RNA (D-RNA) is, but no investigation was made of nucleic acid metabolism.

The Inductive Act. The preceding experiments were done with 2.0-cm segments of corn radicle excised 0.5 cm back of the tip. Is it the washing or the wounding or both which induces the development of increased solute absorption rates?

Excised primary roots were washed whole or cut into 2, 4, or 8 equal segments. The number of cuts or "wounds" has no effect on the increase in phosphate absorption (*e.g.*, absorption was 0.52, 0.48, 0.48, and 0.54 μmole/g fresh weight·hr for intact roots and roots cut into 2, 4, or 8 segments, respectively).

Intact seedlings were lifted off the moist toweling on which they had germinated, and the root systems were submerged in aerated washing medium for 2 hr. After washing, the usual 0.5- to 2.5-cm segments were cut for assay of phosphate uptake rates. The standard procedures were followed for comparison. Washing of intact roots gave the same enhancement of phosphate absorption as did washing of segments (*e.g.*, 88% increase on washing cut sections, 92% on washing intact roots). Wounding is not involved; it is the washing which serves as the inducing act.

What then about roots grown in solution culture—should they not be already induced? In one sense they proved to be, in that freshly excised solution-grown root tissue had rates of absorption comparable to tray-grown or vapor-grown (see "Materials and Methods") roots which had been washed (Table VIII). However, an additional increment of rate could be added by the standard washing procedure. There was no change in respiration rate during washing (just as for tray-grown roots, Table I), and there was no correlation between respiration rates and phosphate absorption rates (Table VIII).

Electron Microscopy. Submersion of roots grown under highly aerobic conditions might restrict the normal rate of oxygen supply (23) and induce synthesis of membranes. Whaley *et al.* (28) have described rapid formation of cytoplasmic membranes in corn roots under conditions of injury or restricted respiration. Van der Heide *et al.* (27) discovered anaerobically grown barley roots to have a higher protein content and a greater capacity for KCl uptake. Jackman and van Steveninck (9) have described changes in the endoplasmic reticulum of beet root slices during washing.

In collaboration with Dr. H. H. Mollenhauer (Charles F. Kettering Research Laboratory, Yellow Springs, Ohio) the effect of washing on subcellular structure was studied with the electron microscope with the use of glutaraldehyde-fixed, uranyl- and lead-stained material. Although of excellent quality, the micrographs showed no detectable change in subcellular structure due to washing and are thus not published.

Tissue Analysis. On the chance that some quantitative change in membranes occurred which would not be detected in the electron micrographs, we analyzed tissue for microsomal protein and total phospholipid (Table IX). No increase due to washing was found.

DISCUSSION

Root tissue proves to be very suitable for studies on the enhancement of solute absorption by washing. It shows no respiratory enhancement or growth upon washing, thus narrowing the field of induced syntheses which are implicated. The response is much more rapid than with stem tissues, thus minimizing any senescence after excision. Indeed, with tray-grown roots one does not even need to excise the roots to ob-

Table VIII. *Effect of Growth Technique on Subsequent Rates of Respiration and Phosphate Absorption of Corn Root Tissue*

Growth Technique[1]	Washing Pretreatment[2]	Oxygen Consumption	Phosphate Absorption
		μl/g fresh wt·hr	*μmoles/g fresh wt·hr*
Tray grown	None	482	0.22
	Warburg flask, 2 hr		0.37
Solution grown	None	599	0.37
	Warburg flask, 2 hr		0.66
Vapor grown	None	622	0.20
	Warburg flask, 2 hr		0.39

[1] See "Materials and Methods."

[2] Tissue was washed in 0.2 mM potassium phosphate and 0.2 mM $CaCl_2$ at 30 C in the Warburg flask while the respiration rate was measured. Respiration rate was linear for the 2 hr. At termination of washing, tissue was removed and ^{32}P uptake was determined by the standard procedure.

Table IX. *Effect of Washing on Lipid Phosphate and Membrane Protein Content of Corn Root Segments*

Washing Pretreatment	Microsomal Protein[1]	Lipid Phosphate	
	mg/g fresh wt	*μmoles/g fresh wt*	*μmoles/mg protein*
None	0.746	1.56	0.286
2 hr, 30 C	0.738	1.57	0.290

[1] Fraction sedimenting between 12,000*g* for 20 min and 80,000*g* for 1 hr.

tain the washing response. The increased phosphate uptake by additional washing of sections from solution-grown roots may, however, involve some reaction to wounding; we have not investigated this.

There seem to be two aspects of the washing response: (*a*) induction by some unknown mechanism activated by submersion and (*b*) development by processes dependent on aerobic metabolism and RNA and protein synthesis.

The physiology and biochemistry of induction are obscure. Approximately 30 min of lag period are involved. The work of Macklon and Higinbotham (16) with pea epicotyl shows the lag period to be occupied by a rapidly increasing electropotential difference between the vacuole and external medium. A similar rapid development of negative cell potential occurs in excised, solution-grown barley roots (22). The fact that the cell potential starts rising immediately suggests that induction may be linked to this phenomenon. Pitman *et al.* (22) speculate that the rising potential in their experiments is probably due to excision of the hormonal supply from the discarded root tip or to healing of exposed and leaky plasmodesmata. These explanations will not serve for the positive response we obtained by washing intact roots.

Induction might be due to leaching of an inhibitory substance, as suggested for sliced storage tissue (11, 12), or release of an activating agent (20). Restriction of the rate of oxygen supply for respiration consequent to submersion (23) might alter levels of some critical metabolite or hormone. The antagonistic action of kinetin or auxins suggests a hormonal basis, but the similar action of 2,4,6-T imposes caution.

The development of the enhanced solute transport rate is more susceptible to experimental attack than induction. The inhibitor studies made here give the same result obtained by others (see the introduction): increased absorption rates with washing appear to depend on the synthesis of new proteins. Presumably induction triggers the synthesis of enzymes implicated in solute transport. What is needed here is the identification of a "transport enzyme" with an increase that can be followed during washing. In the accompanying paper we describe our investigation of what is presumably one such enzyme.

Pitman (21) has commented on the adaptation of roots to changes or differences in environment. The results obtained here permit some amplification. During periods of high soil water content a situation simulating "washing" may prevail. Induction and development of higher salt absorption rates would partially compensate for restricted gas exchange, enabling the plant to maintain adequate mineral nutrition. In the course of evolution this process may have had survival value, selecting out root systems which respond rapidly.

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