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Adenine Nucleotide Content of Corn Roots as Affected by Injury and Subsequent Washing[1]

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ABSTRACT

The adenine nucleotide content of the 2-centimeter segments excised from tray-grown corn (*Zea mays* L., WF9 × Mo17) roots declines for the first hour after excision. Concomitant with the loss of adenine nucleotides is a decline in respiration and a leakage of K^+. With continued washing, these parameters partially or completely recover and increased phosphate influx develops. Increasing the wound effect by cutting 0.5-centimeter segments gives a more rapid and pronounced degradation of adenine nucleotides and slower recovery. Conversely, the mild injury caused by submerging intact roots induces less degradation and produces greater net adenine nucleotide synthesis during recovery; adding auxin to the washing medium produces a similar result. With all treatments, there is stabilization of energy charge at about 0.85.

Brief submersion or rubbing of intact roots, as well as recutting washed and recovered root segments, will initiate the transient loss of adenine nucleotides but will not induce increased phosphate influx.

It is suggested that the loss in adenine nucleotides may reflect homeostasis in energy charge via catabolism arising from membrane permeability changes.

Washing (or 'aging') of excised root tissue leads to increased ion influx (9, 10, 13, 16, 24), H^+ efflux (13, 14), and electrogenic cell potential (14, 20). To a large extent these changes reflect recovery from injury inflicted by cutting, cold shock, or handling the tissue (10, 13, 14). There are two phases to the washing response: an initial phase lasting about 1 h during which there is rapid (no lag) reinitiation of the H^+ and K^+ fluxes and cell potential, much as with fusicoccin but slower, followed by a general augmentation of solute transport, typified by increase in phosphate influx and microsomal K^+-ATPase activity (14, 16, 17). These phases have been termed 'inductive' and 'developmental' (14, 16).

During the inductive phase, there is a decline in the level of ATP which recovers if washing is continued for 3 to 4 h (19). Similar changes in adenine nucleotides (and other metabolites) during washing or aging of excised plant tissue have been reported by other investigators (7, 21, 23, 25). The phenomenon must reflect some important metabolic change in the cells which is linked to wounding or other injury, and in corn roots may have a role in inducing the developmental phase.

In the study reported here, we have examined the changes in AdN[3] content of excised corn root tissue in relation to tissue respiration, K^+ content, and phosphate influx. Cutting and other types of injury or stress have been compared. We conclude that injury stimuli induce membrane 'leakiness,' which indirectly leads to net loss of AdN. Inasmuch as energy charge is relatively stable, it is probable that the loss is initiated by rapid ATP hydrolysis followed by AMP degradation. There is no evidence that the increased phosphate absorption derives from the transient decline in AdN concentration.

A preliminary report on part of this work has been given (12).

MATERIALS AND METHODS

Plant Material and Treatments. Corn seeds (*Zea mays* L., WF9 × Mo17) were germinated for 3 d in the dark at 28°C on paper towels saturated with 0.1 mM $CaCl_2$, and 2-cm segments were excised 0.5 to 2.5 cm from the tip of the primary root as described (16). Segments were assayed for AdN or phosphate influx immediately after cutting, and after various periods of washing in aerated 0.2 mM $CaCl_2$ + 0.2 mM KH_2PO_4 (adjusted to pH 6.0) at 30°C (16). In some experiments, the 2-cm segment was additionally wounded by cutting into four 0.5-cm segments (13). Where the effect of excision without washing was examined, the segments were aged on paper towels under the same conditions in which they had been grown. Washing of intact roots was done by transferring tray-grown intact plants to a screen support with roots submerged in the washing medium. Segments were excised at the time of assay.

Additional injury treatments were given by lifting the seedlings from the trays and either submerging them for 2 min in aerated 0.1 mM $CaCl_2$ at 28°C (brief submersion), or gently stroking the roots from base to apex between thumb and forefinger five times (rubbing). The intact plants were then returned to growth conditions with segments taken for assay at stated periods.

Phosphate Influx. Phosphate absorption rates were determined by incubating the tissue at 30°C for 30 min in the standard washing solution labeled with ^{32}P as described previously (14).

K^+ Content. K^+ was determined by flame photometry on acid digests of the root tissue.

Determination of AdN. Twenty segments were dropped into 10 ml of boiling 50 mM Hepes buffer, pH 7.5. After boiling for 1 min, the tissue and extract were cooled in an ice bath, homogenized, and the extract was clarified by centrifugation. ATP was determined on 100-μl aliquots of extract, using an Aminco-Chem Glow photometer and 50 μl of reconstituted luciferin-luciferase (Sigma, FLE-50). AdN were determined by incubating 0.2 ml of extract in the following mixtures for 15 min at 30°C prior to assay for ATP: (a) for ATP, 0.2 ml of buffer (50 mM Hepes + 50 mM Mg acetate, pH 7.5) and 0.2 ml distilled H_2O; (b) for ATP plus ADP, 0.2 ml buffer and 0.2 ml of solution containing 10 μg pyruvate kinase

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[3] Abbreviation: AdN, adenine nucleotide(s).

plus 1 μmol trisodium phosphoenolpyruvate (Sigma); (c) for total AdN, 0.2 ml of buffer and 0.2 ml of solution containing 10 μg adenylate kinase (dialyzed against 1 mM K-phosphate, pH 7.0) in addition to pyruvate kinase and phosphoenolpyruvate as above.

Respiration. Respiration was determined with a Clark O_2 electrode at 30°C in 10 ml of air-saturated washing solution. To accommodate the vessel, it was necessary to cut the 2-cm segments into 1-cm segments. This was done prior to washing.

RESULTS

AdN Content and K^+ Leakage. Figure 1A verifies the report (19) that ATP falls for about 1 h after excision, but recovers with continued washing. Total AdN content follows a similar pattern, demonstrating initial net loss followed by net synthesis in the developmental phase. The net gain during recovery is in ATP; ADP and AMP decline following injury and do not recover. As a consequence, energy charge is high and rises slightly during washing. Energy charge was found to be remarkably stable, varying from about 0.80 to 0.85 over the washing period. Bieleski (1), drawing on available analyses, concluded that plant tissues have average ratios of 10 ATP:3 ADP:1 AMP for an energy charge of 0.82.

Figure 1B shows the associated pattern of increased phosphate influx after an initial lag as described by Leonard and Hanson (16). They also reported an initial K^+ loss followed by net gain, as have Smith and Harper (27). Figure 1B shows the time course of this phenomenon, which is often observed in excised tissue (28). There is a strong parallel in the loss and initiation of recovery in K^+ and ATP content. If allowance is made for the 30-min period for determining phosphate absorption, the lag period ends at about the same time as the loss of K^+ and ATP stops.

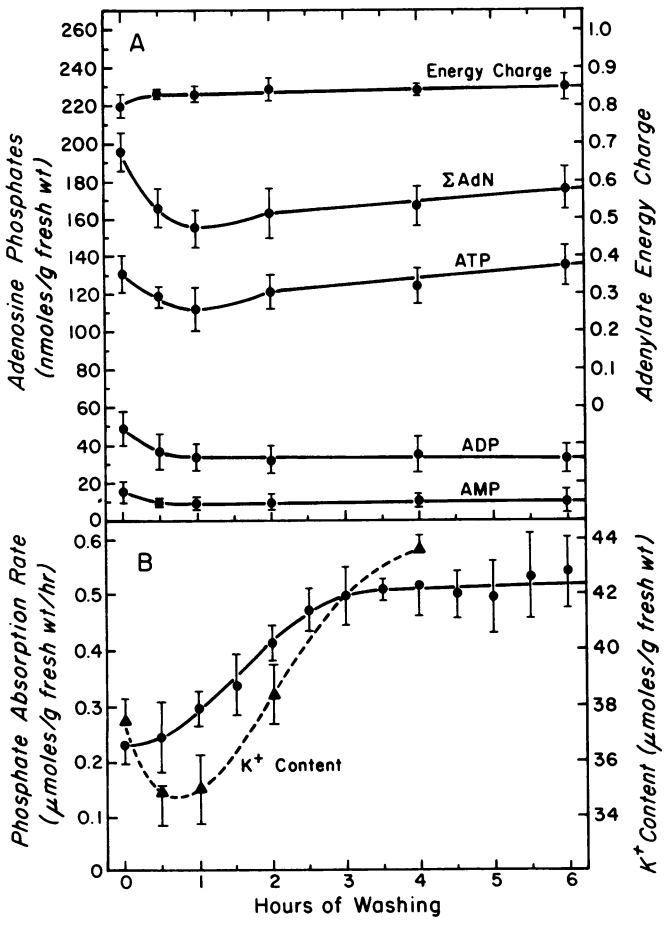

FIG. 1. AdN content, energy charge, phosphate absorption rate, and K^+ content of 2-cm segments of tray-grown corn roots during washing. Means ± SD. See "Materials and Methods" for details.

Since K^+ leaks from the injured tissue, it is possible that AdN may be lost in the same way. However, very little AdN could be detected in the washing medium after 1 h, corresponding to no more than 18% of the tissue loss. It is possible, of course, that the AdN were degraded beyond AMP, and thus escaped detection by the luciferin-luciferase assay. Cell walls contain phosphatase activity (1).

AdN are not the only nucleotides to decline during the first h of washing. UDPG, the other major acid soluble nucleotide of corn roots (6), declines about 30% (11). Collins *et al.* (7) have reported a decline and subsequent recovery in UDP-sugars in excised barley aleurone layers.

Respiration. Although comparisons of fresh *versus* washed tissue respiration show no significant differences (14, 16), Lin (18), in a more detailed study, found about 10% decline in respiration during the first h, which was largely recovered by 4 h. There was also an increase in the cyanide-insensitive respiration. In the present experiments, the decline in respiration was about twice as large and the recovery was incomplete (Fig. 2). Cyanide-insensitive respiration rose rapidly in the first h, less so thereafter. It is interesting that the potential for cyanide-insensitive respiration becomes high during the second or developmental phase of the washing response, but the significance of this is unknown.

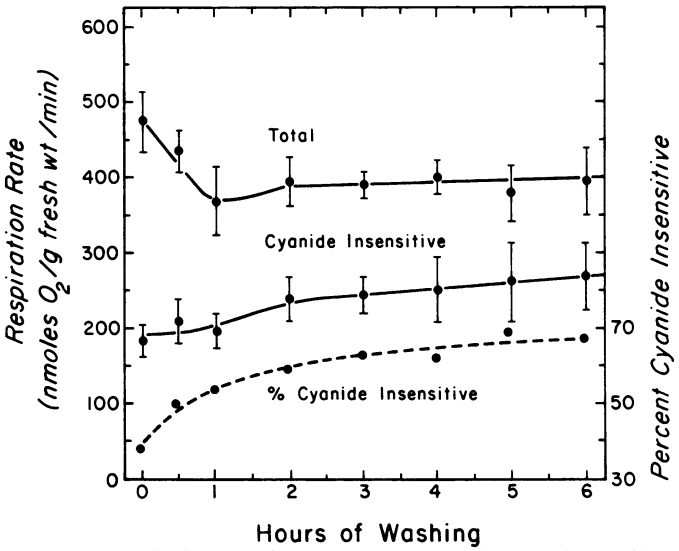

FIG. 2. Respiration rate of 1-cm segments of root tissue during washing. Cyanide-insensitive respiration is that in the presence of 1 mM KCN.

It appears that the initial depression of respiration is probably linked to the loss of AdN.

Other Injury or Handling Responses. We reported previously (13) that increasing the extent of injury by cutting the standard 2-cm segment into four 0.5-cm pieces increased the initial drop in H^+ efflux and K^+ and Cl^- influx, and lengthened the period of recovery. Initial phosphate influx was not affected, but, as with 2-cm segments, increased phosphate absorption rates developed. Figure 3 gives the corresponding comparison for AdN content of 2- and 0.5-cm segments. The greater proportion of wounding in the 0.5-cm segments leads to a low initial level of ATP and a low energy charge, which requires 2 h for recovery. The initial degradation of the ATP occurs during the time it takes to cut and weigh the segments, about 5 min.

The relationship between the drop in AdN levels and the development of increased phosphate influx was explored. Submersion of intact, tray-grown (highly aerobic) roots is sufficient to induce enhanced phosphate absorption rates (16); does submersion also initiate the transient decline in AdN content? As shown in

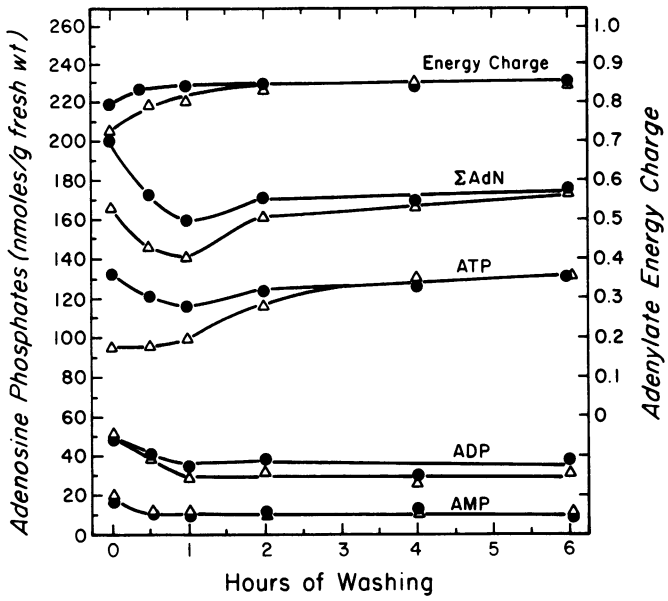

FIG. 3. Comparison of standard 2-cm root segments with 0.5-cm segments. Experimental conditions as in Figure 1. (●), 2-cm segments; (△), 0.5-cm segments.

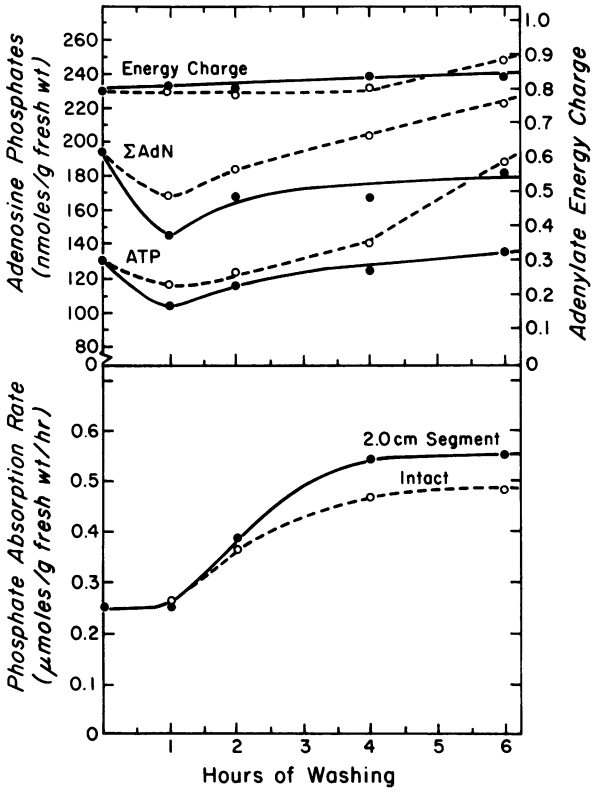

FIG. 4. Comparison of washing responses in standard 2-cm segments with those of roots of intact plants. Corresponding 2-cm segments of intact roots were cut at the indicated times of assay. (——), 2-cm segments; (- - -), intact roots. Experimental conditions as in Figure 1.

Figure 4, it does. However, the decline in AdN is less extensive in the intact roots, and the recovery during the developmental phase is of greater magnitude, possibly because of the greater availability of metabolites (and hormones?) in the intact seedling. Using 0.5-cm root tips, Saglio and Pradet (25) found addition of glucose to the medium necessary for stable recovery of AdN and respiration.

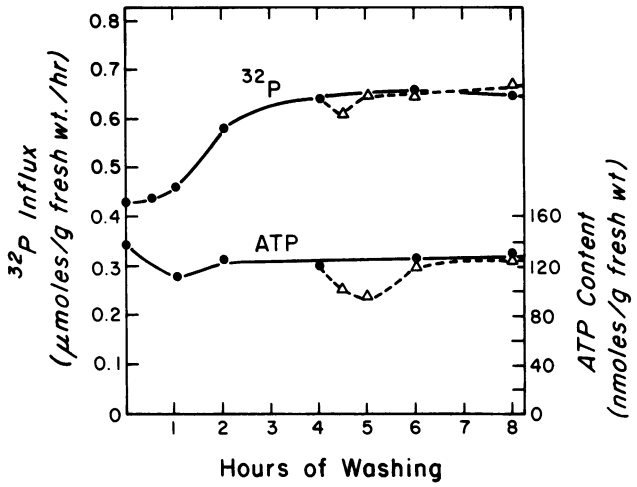

FIG. 5. Effect of reinjury on ATP content and phosphate influx. After 4 h washing, the 3-cm root segments were cut into 1-cm segments. (——), control segments; (- - -), cut segments. Experimental conditions as in Figure 1.

Table I. *Effect of Mechanical Injury on ATP Content and Phosphate Uptake by Corn Roots*

Control refers to 2-cm segments washed as in Figure 1. Rubbing and brief submersion (2 min) were treatments of intact roots which were subsequently aged on wet toweling with 2-cm segments excised at time of assay (see "Materials and Methods").

Time of Washing or Aging	Treatment					
	Control		Rubbing		Brief submersion	
	ATP[a]	Pi[b]	ATP	Pi	ATP	Pi
h						
0	131	0.29	123		129	
1	112	0.27	106	0.20	119	0.20
4	124	0.63	136	0.17	134	0.19

[a] ATP content, nmol/g fresh weight.
[b] ^{32}P-phosphate influx, μmol/g fresh weight·h.

It was found that aging 2-cm segments on saturated paper toweling at 28°C gave the same changes in AdN content and phosphate influx as did the standard washing procedure (data not shown).

Figure 5 shows that recutting 4-h washed and recovered tissue acts to renew the cycle of ATP loss and recovery. Phosphate absorption, on the other hand, shows only a small dip.

Table I gives data from experiments in which injury stimuli were even more moderate. Controls were the standard 2-cm segments washed in the usual fashion (*e.g.* Fig. 1). For the treatments, intact roots were briefly submersed or rubbed and then returned to conditions under which they were grown (toweling saturated with 0.1 mM $CaCl_2$), with the 2-cm segments excised at time of assay (*i.e.* the intact roots were 'aged' rather than 'washed'). The interesting result here is that, although gentle rubbing or 2 min submersion will trigger the transitory decline in ATP content, these stimuli do not initiate the development of phosphate absorption. This finding, and that of Figure 5, shows that the change in ATP content cannot be the signal that induces the developmental changes. There may be a quantitative aspect such that injury or shock must be extensive if development of increased phosphate absorption is to occur (*e.g.* continuous submersion, as in Fig. 4, compared with 2 min submersion in Table I).

Other evidence that changes in AdN levels are independent of phosphate influx is found with auxin treatment. Washing root

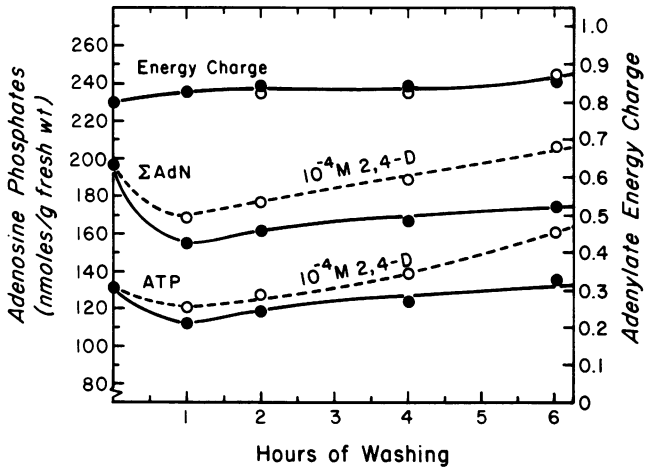

FIG. 6. Effect of adding 0.1 mM 2,4-D to the washing solution on the AdN content and energy charge of 2-cm segments. Experimental conditions as in Figure 1.

segments in the presence of 0.1 mM IAA or 2,4-D for 2 h will decrease phosphate absorption by 50 to 60% (16), but, of the phosphate absorbed, there is 3-fold increased incorporation into nucleic acids (17). It is known that treatment of pea stem segments with IAA (22) and soybean hypocotyl segments with 2,4-D (15) will increase the ATP content. Figure 6 shows the effect of washing segments with 2,4-D on the levels of AdN. Although the initial decline in AdN is less in the presence of auxin, it still occurs. The recovery is more pronounced and resembles that of intact roots (*cf.* Fig. 4), suggesting that one limitation on AdN biosynthesis during recovery of excised tissue may be hormonal. The important point here is that a treatment known to inhibit phosphate absorption actually promotes AdN recovery.

DISCUSSION

It is clear that various stimuli, some quite gentle, will initiate a transitory loss of AdN in corn roots. Saglio and Pradet (25) reported recently that total AdN content of excised corn root tips drops during the early stages of aging. Excised pea stem segments initially lose nucleotides and then regain them (23). Excision of barley aleurone layers leads to an initial decline in ATP content which recovers with washing (7). In freshly excised discs of tobacco leaves, the ATP content declines for the first 5 to 10 min, but recovers thereafter (21). It appears that the phenomenon is a general one, and that AdN extractions must be made rapidly with a minimum of injury or 'shock' if the determinations are to reflect normal metabolic conditions accurately.

The concentration of AdN might be reduced by biosynthetic events, such as in the synthesis of polysomes initiated by wounding (8). However, about 15 min elapses after cutting pea epicotyls before an increase in polysomes can be detected, and this initial increase is at the expense of preexisting monosomes and mRNA (8). About 1 h elapses before there is significant net synthesis of rRNA. In corn roots, on the other hand, the loss of AdN starts immediately, and in the 0.5-cm segments there is a sizeable decline during the 5 min required to cut and weigh the segments (Fig. 3).

An alternative is that the net loss of AdN reflects homeostasis of energy charge via catabolism. Mammalian tissues and microorganisms stabilize energy charge under stress by regulating AdN pool size (3, 4, 26). If corn root tissue reacts in a similar fashion injury should increase ATP hydrolysis, producing ADP and AMP, followed by rapid dephosphorylation and/or deamination of AMP, thus reducing total AdN. Adenylic kinase would maintain a high proportion of ATP and high energy charge (2).

For this scheme to be valid, the injury or shock must activate or release degradative enzymes not normally active *in vivo*. Bieleski (1) concludes most of the high level of phosphatase activity of plant cells is sequestered in the vacuole away from substrates, although some activity resides in the walls. Thus, increased permeability of the tonoplast and/or plasmalemma would be required for the catabolism. Present evidence for a permeability change with injury or shock is limited to net K^+ efflux (Fig. 1), H^+ influx (5), and Ca^{2+} influx (G. Zocchi, unpublished experiments). All of these fluxes involve the plasmalemma, but the 5 to 10% loss of K^+ suggests efflux from the vacuole as well, and increased tonoplast permeability is likely.

The rate of AdN loss slows after 30 min, and by 60 min net recovery is initiated (Fig. 1). It appears that the induction of increased AdN biosynthesis to offset catabolism must start soon after wounding or shock, with a clearly discernable effect about the time net K^+ loss stops and the increase in phosphate absorption begins. As yet, there is little evidence on the interrelationships of these processes except that induction of enhanced phosphate absorption is not so responsive to injury as is AdN content (Fig. 5; Table I). The recent report of Davies and Schuster (8) suggests to us that increases in polysomes and protein synthesis underlie the developmental changes in corn root tissue, since these changes are blocked by inhibitors of RNA and protein synthesis (16).

The recovery in AdN content is higher in intact (Fig. 4) and auxin-treated (Fig. 6) tissue, for which there is precedent (see comments in text). The auxin experiments were limited to determining if any linkage exists between the increases in AdN content and phosphate absorption, and no conclusion about auxin action can be drawn.

In conclusion, the data suggest that various injuries or shocks may effect membrane permeability changes which lead to transitory catabolism of AdN in a fashion which maintains energy charge. After a lag, there is induction of AdN biosynthesis which completely recovers AdN concentrations, conditioned to some degree by metabolite and/or hormone supply. At present, the only observed physiological correlate with lowered AdN content is a lowered respiration rate.

LITERATURE CITED

1. BIELESKI RL 1973 Phosphate pools, phosphate transport, and phosphate availability. Annu Rev Plant Physiol 24: 225–252
2. BOMSEL J-L, A PRADET 1968 Study of adenosine-5′-mono-, di- and triphosphates in plant tissues. IV. Regulation of the level of nucleotides, *in vivo*, by adenylate kinase: theoretical and experimental study. Biochim Biophys Acta 162: 230–242
3. CHAPMAN AG, DE ATKINSON 1973 Stabilization of adenylate energy charge by the adenylate deaminase reaction. J Biol Chem 258: 8309–8312
4. CHAPMAN AG, L FALL, DE ATKINSON 1971 Adenylate energy charge in *Escherichia coli* during growth and starvation. J Bacteriol 108: 1072–1086
5. CHASTAIN CJ, JB HANSON 1982 Control of proton efflux from corn root tissue by an injury-sensing mechanism. Plant Sci Lett 24: 97–104
6. COLE CV, C ROSS 1966 Extraction, separation, and quantitative estimation of soluble nucleotides and sugar phosphates in plant tissues. Anal Biochem 17: 526–539
7. COLLINS GG, CF JENNER, LP PALEG 1972 The levels of soluble nucleotides in wheat aleurone tissue. Plant Physiol 49: 398–403
8. DAVIES E, A SCHUSTER 1981 Intercellular communication in plants. Evidence for a rapidly generated, bidirectionally transmitted wound signal. Proc Natl Acad Sci USA 78: 2422–2426
9. FRICK H, RL NICHOLSON, LF BAUMANN 1977 Characterization of augmented potassium uptake in the seedling primary root of inbred maize (*Zea mays*). Can J Bot 55: 1128–1136
10. GLASS ADM 1978 Influence of excision and aging upon K^+ influx into barley roots. Recovery or enhancement? Plant Physiol 61: 481–483
11. GRONEWALD JW 1979 The effect of injury on transport processes in corn root tissue. PhD thesis. University of Illinois, Urbana-Champaign
12. GRONEWALD JW, JB HANSON 1976 Effect of stress on the adenine nucleotide content of maize root tissue. Plant Physiol 57: S–92
13. GRONEWALD JW, JB HANSON 1980 Sensitivity of the proton and ion transport mechanisms of corn roots to injury. Plant Sci Lett 18: 143–150
14. GRONEWALD JW, JM CHEESEMAN, JB HANSON 1979 Comparison of the responses of corn root tissue to fusicoccin and washing. Plant Physiol 63: 255–259
15. KEY JL, JB HANSON 1961 Some effects of 2,4-dichlorophenoxyacetic acid on soluble nucleotides and nucleic acid of soybean seedlings. Plant Physiol 36:

145–152
16. LEONARD RT, JB HANSON 1972 Induction and development of increased ion absorption in corn root tissue. Plant Physiol 49: 430–435
17. LEONARD RT, JB HANSON 1972 Increased membrane-bound adenosine triphosphatase activity accompanying development of enhanced solute uptake in washed corn root tissue. Plant Physiol 49: 436–440
18. LIN W 1976 Ion transport and cell potentials in corn root tissue. PhD thesis. University of Illinois, Urbana-Champaign
19. LIN W, JB HANSON 1974 Phosphate absorption rates and adenosine-5′-triphosphate concentrations in corn root tissue. Plant Physiol 54: 250–256
20. LIN W, JB HANSON 1974 Increase in electrogenic membrane potential with washing of corn root tissue. Plant Physiol 54: 799–801
21. MACNICOL PK 1976 Rapid metabolic changes in the wounding response of leaf discs following excision. Plant Physiol 57: 80–84
22. MARRÉ E, G FORTI 1958 Metabolic responses to auxin. III. The effects of auxin on ATP level as related to the auxin induced respiration increase. Physiol Plant 11: 36–47
23. PALMER JM, BC LOUGHMAN 1964 Some factors affecting the rate of absorption and utilization of ions in stem segments. New Phytol 63: 217–231
24. PARRONDO RT, RC SMITH 1976 Effect of removal of the root tip on the development of enhanced Rb^+ absorption by corn roots. Plant Physiol 51: 378–381
25. SAGLIO PH, A PRADET 1980 Soluble sugars, respiration, and energy charges during aging of excised maize root tips. Plant Physiol 66: 516–519
26. SCHRAMM VL, H LEUNG 1973 Regulation of adenosine monophosphate levels as a function of adenosine triphosphate and inorganic phosphate. A proposed metabolic role for adenosine monophosphate nucleosidase. J Biol Chem 248: 8313–8315
27. SMITH RC, JC HARPER 1977 Influence of the root tip and the duration of washing on K^+ retention by excised apical root segments of corn. Plant Physiol 59: 335–336
28. VAN STEVENINCK RFM 1975 The "washing" or "aging" phenomenon in plant tissues. Annu Rev Plant Physiol 26: 237–258