Plant Physiol. (1989) 90, 53–58
0032-0889/89/90/0053/06/$01.00/0

Received for publication July 6, 1988
and in revised form December 19, 1988

Effects of NaCl on Metabolic Heat Evolution Rates by Barley Roots

Richard S. Criddle, Lee D. Hansen[1], R. William Breidenbach, Michael R. Ward[2], Ray C. Huffaker*

Department of Biochemistry and Biophysics (R.S.C., L.D.H.) *and Plant Growth Laboratory* (R.W.B., M.R.W., R.C.H.), *University of California, Davis, California 95616*

ABSTRACT

The effect of salinity stress on metabolic heat output of barley (*Hordeum vulgare* L.) root tips was measured by isothermal microcalorimetry. Several varieties differing in tolerance to salinity were compared and differences quantified. Two levels of inhibition by increasing salt were found. Following the transition from the initial rate to the first level, inhibition remained at about 50% with further increases in salt concentration up to 150 millimolar. The concentration of salt required to inhibit to this level was cultivar dependent. At higher concentrations (>150 millimolar) of salt, metabolism was further decreased. This decrease was not cultivar dependent. The decreased rate of metabolic heat output at the first transition could be correlated with decreases in uptake of NO_3^-, NH_4^+, and Pi that occurred as the salt concentration was increased. The high degree of dependence of the inhibition of metabolic heat output on NaCl concentration points to a highly cooperative reaction responsible for the general inhibition of metabolism and nutrient uptake. The time required to attain the first level of salt inhibition is less than 20 minutes. Inhibition of root tips was not reversible by washing with salt free solutions. In addition to revealing these features of salt inhibition, isothermal microcalorimetry is a promising method for convenient and rapid determination of varietal differences in response to increasing salinity.

Although inhibition of plant growth by elevated levels of NaCl has been studied extensively, the biochemical mechanisms and energy relationships involved in this inhibition have not yet been clearly defined.

Root respiration accounts for an appreciable amount of total plant respiration. For a corn plant growing under normal conditions, about 50% of the total respiratory energy of the roots is consumed by ion uptake and roughly 20% of the total respiration of the entire plant is used for uptake and transport of ions (20). Since ion uptake requires both high energy phosphate and electron flow (13), the energy considerations for ion uptake are of primary importance.

Several studies have suggested that NaCl effects on metabolite uptake may explain some of the major salt effects (1, 15, 21, 22). Concentrations of NaCl inhibiting plant growth correlate closely with those inhibiting uptake of key metabolites (21, 22). Moreover, NaCl inhibition curves for uptake of NO_3^-, Pi, and NH_4^+ are virtually identical for barley seedlings (22). These results suggest that the effects of high NaCl on transport are general.

Protective effects of divalent cations on NO_3^- uptake in the presence of NaCl have led to suggestions that membrane structure and function are altered by high levels of salt (20). For example, Cramer *et al.* (3) found that increasing concentrations of Ca^{2+} in saline solutions helped maintain K/Na selectivity by cotton roots. Cramer *et al.* (2) showed that high NaCl concentrations displaced Ca^{2+} from the plasmalemma of cotton roots and increased efflux of cytosolic K^+. Lynch and Laüchli (14) recently showed that high NaCl displaces Ca^{2+} associated with intracellular membranes.

We have now employed isothermal microcalorimetric measurements of metabolic heat evolution to allow determination of the changes in metabolic rates of root tissues stressed with varying levels of NaCl. Concentrations of NaCl in the range that inhibit metabolic heat rates also inhibit nutrient uptake and plant growth. Effects of metabolic poisons and divalent cations on NaCl inhibition of metabolism were examined to further define metabolic pathways subject to NaCl inhibition.

MATERIALS AND METHODS

Plant Materials

Seeds of barley (*Hordeum vulgare* L.) cultivars CM 72 and Numar with moderate salt tolerance, and the lower salt tolerant Arivat were used (5). Seeds were surface-sterilized for 10 min in 0.25% sodium hypochlorite, rinsed with water, and germinated at 25°C between sheets of Whatman No. 1 filter paper soaked with ¼ or in some cases 1/20 strength Hoagland solution (pH 5.9). Root tips were collected from 3- to 4-d-old seedlings by cutting approximately 1 cm sections with a razor blade. The root tips were kept moist in the diluted Hoagland solution or in some cases were incubated for 1 h in 10 mM $CaSO_4$ prior to use.

Calorimetry Measurements

Root tips from about 10 plants weighing approximately 100 mg were placed on a Whatman GFC filter disk in the bottom of the sample ampules of a Hart Scientific model 7707 differential scanning calorimeter. The sample and disk were washed three times with the appropriate test nutrient

[1] Visiting Professor from the Department of Chemistry, Brigham Young University, Provo, UT.

[2] Present address: Department of Genetics, Harvard Medical School and Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114.

solution and left with sufficient solution to wet the filter disk and tissues (200 μL) when the ampule was sealed. Metabolic heat rates were measured using the isothermal operating mode of the calorimeter at 23°C. Heat rate measurement was made continuously until a constant heat flow rate indicated that the sample and chamber had attained a steady state (approximately 45 min). The ampules were then opened, the samples were washed three times and equilibrated with the next desired solution, and the heat rate measurement was repeated. Three tissue samples could be examined simultaneously from three ampules using this procedure. The heat rates obtained are a measure of the metabolic rates (4).

Treatments with Inhibitors and Protective Substances

Metal ions or inhibitors were added to the wash solution and the roots were washed three times, after which heat rates were determined. In tests of the reversibility of salt inhibition, roots were treated with 150 mM NaCl for varying lengths of time, then washed three times with 1 mL of nutrient solution free of salt and containing appropriate test substances. These treated and washed samples were then placed in the calorimeter.

Effect of NaCl on NO_3^-, NH_4^+, and Pi Uptake

Barley seedlings (CM 72) were grown hydroponically for 7 d in darkness as described (7, 21). Seedlings were then transferred to continuous light growth conditions for 3 d in aerated one-quarter strength Hoagland solution (8) for the Pi uptake experiments and in one-quarter strength N-free (8) Hoagland solution for the NH_4^+ and NO_3^- uptake experiments. Seedlings used in the NH_4^+ and NO_3^- uptake experiments were supplied 1 mM NH_4HCO_3 or KNO_3 for 24 h, respectively, to fully induce the transport systems (7). Nutrient solutions for measurement of NO_3^- and NH_4^+ transport contained one-quarter strength Hoagland solution lacking N, 1 mM Mes to maintain the pH between 6.2 and 6.5, and either 1 mM KNO_3 or NH_4HCO_3. Phosphate uptake was measured using a one-quarter strength Hoagland solution lacking P with added 1 mM K-phosphate (pH 6.2) and 1 mM Mes. Sodium chloride was added to the uptake solutions at concentrations up to 150 mM. Uptake of NO_3^- and NH_4^+ were determined by following the depletion of the solutions at 2 h intervals for 12 h. Nutrient solutions were replaced periodically to prevent greater than 20% depletion of the initial concentration. Phosphate uptake was determined over a 10 h absorption period.

Analysis of NO_3^-, NH_4^+, and Phosphate

Nitrate and NH_4^+ were determined as described previously (6, 19). Phosphate was determined by the method of Murphy and Riley (16).

RESULTS

Figure 1 shows the time course of a typical measurement of the metabolic heat rate of CM 72 barley root tips. The large initial transient heat flows reflect those occurring while the calorimeter and sample approach the thermal steady state.

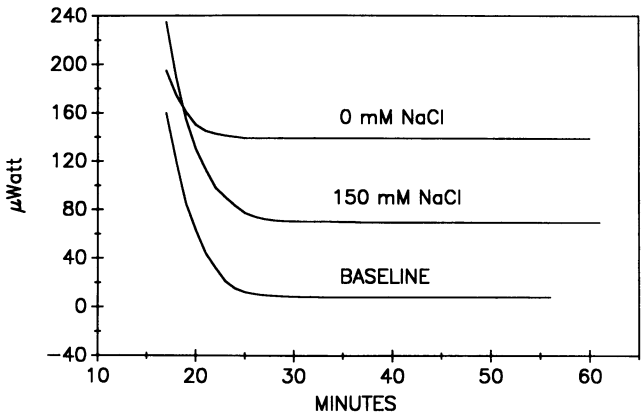

Figure 1. Measurement of metabolic heat rates at 23.987°C by isothermal calorimetry. The initial transient changes in heat represent thermal equilibration of samples and chamber. The steady state heat rates at times greater than about 35 min represent rates of metabolic heat evolution by sample root tissues. The two upper curves show heat rates of a single sample of CM 72 root tips incubated with no added NaCl (top) and then at 150 mM NaCl (center curve). The differences between the steady state rates of these two curves is a measure of salt inhibition of metabolism. The lower curve is a baseline measurement of the empty calorimeter ampule without sample.

Later, as the curve flattens and the heat rate becomes constant, the actual steady state rate of metabolic heat production is observed. These steady state heat rates (μW) are stable for several hours, remaining essentially constant until O_2 in the chamber is depleted (ref. 4 and unpublished results for barley roots). The lowered rate of heat evolution from salt treated roots compared to untreated roots represents the salt inhibition of the metabolic rates. The observed heat rate (μW) depends upon the mass of tissue as well as the metabolic rate. Consequently, the results for each test sample must be expressed as either the heat output rate per g of tissue, or as changes relative to a control. In the experiments presented here, it was usually possible to use the same tissue sample, measured just prior to addition of the test substances, as the control. Any changes noted are then due only to changes in metabolic heat rate. Heat rates were routinely 1.5 ± 0.2 μW/mg fresh weight of 3-d-old barley root tissue.

To evaluate whether wounding responses in the cut tissues contributed appreciably to the measured heat rate values, some of the barley root tips were incubated in 1 mM $CaSO_4$ for up to 1 h prior to placement in the calorimeter for heat rate measurement. No effects of preincubation were found. Wounding contributions to the metabolic heat release were either completed prior to the steady state heat rate measurements in this study or were too small to be measured under these conditions.

The responses to stepwise increases in the concentration of NaCl bathing the tissues of three samples of CM 72 root tips, germinated and grown in 1/20 Hoagland solution, are seen in the upper curve of Figure 2. As the roots were exposed to successively increased levels of NaCl, the metabolic heat rates showed an initial increase, followed by a sharp decrease. At 90 mM NaCl, the heat rate was reduced to 50% of the maximum value. Between 90 and 150 mM NaCl, no further

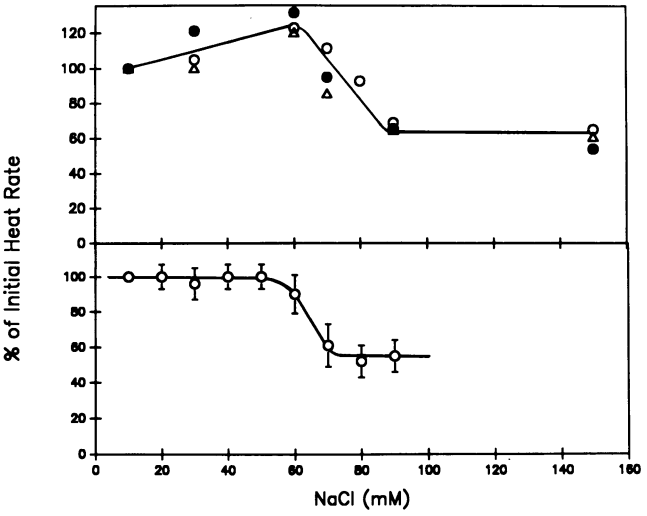

Figure 2. Metabolic heat rates of CM 72 root samples as a function of NaCl concentration. The top curve shows salt titration of roots from plants germinated and grown in ¼ Hoagland solution. Values plotted represent the average values and standard deviation of seven test runs. The lower curve represents values for three root samples, from plants germinated and grown in 1/20 Hoagland solution. To facilitate comparisons of samples with different tissue masses and metabolic heat rates, data are plotted as percent of maximum heat rate.

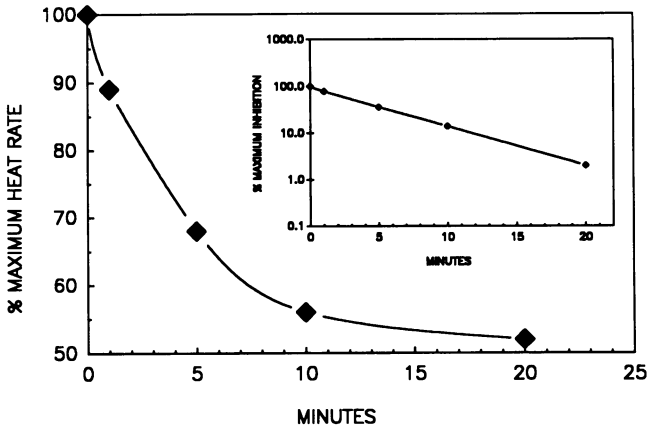

Figure 3. Time course of inhibition of root metabolism by 150 mM NaCl. Maximum inhibition was 50% of total heat rate. Activity at 100% = 128 μW/85 mg of fresh weight roots.

changes in metabolic rates were noted. The increased rates of heat output with low levels of NaCl indicate a salt stimulation of metabolic heat rates. The data plotted in the lower curve in Figure 2 repeat the experiments shown in the upper curve except that the seven samples of root tips were from plants germinated and grown in ¼ strength Hoagland solution. In this case, the increase in metabolic rate induced at low levels of NaCl was not observed and the concentration of NaCl at which 50% inhibition occurred was about 70 mM.

The time course for inhibition of metabolic rates of CM 72 roots by 150 mM NaCl is illustrated in Figure 3. Root samples were immersed in 150 mM NaCl combined with one-eighth Hoagland solution, and incubated for varied time periods before washing exhaustively with salt-free nutrient solution and measuring their metabolic rates with the calorimeter. Decreases occurred within 1 min and nearly 90% inhibition of the salt-sensitive portion of root metabolism was achieved within 10 min of exposure to 150 mM NaCl. Half-time for inhibition under these conditions was about 3.5 min (see inset of Fig. 3). Inhibition was complete by 20 min. Inhibition studies conducted in this fashion measure residual metabolic heat rates at 150 mM NaCl plus any activity recovered after removal of NaCl. However, there is no upward slope during the 20 to 30 min of the measurement to suggest that recovery occurs.

Figure 4 shows that the steady state rates of metabolism of three barley cultivars had different sensitivities to added NaCl at up to 150 mM salt, but responded similarly at higher salt. The less salt-tolerant Arivat roots were strongly inhibited at 20 mM NaCl while CM 72 and Numar were inhibited only at NaCl concentrations in excess of 60 and 100 mM, respectively. Inhibition of metabolism by NaCl was only partial; in all cases, about 50% of the initial metabolic heat rate was retained. At NaCl levels above 200 mM, a second decrease in metabolic heat rates occurred that plateaued at about 20% of the original activity. No differences among the cultivars were detected at the higher salt concentrations.

Figure 5 shows the effects of increasing NaCl concentrations on the rates of NO_3^-, NH_4^+, and Pi uptake by CM 72 barley seedlings. Decreased uptake was detected at 100 to 150 mM NaCl.

The effects of various inhibitory substances on root tip metabolism are presented in Figure 6. All heat rates are plotted as percentage inhibition relative to the heat rates of the test tissue immediately prior to addition of inhibitor. Each column necessarily presents data from a fresh sample of tissue. Column 1 shows a control test with nothing added to the one-

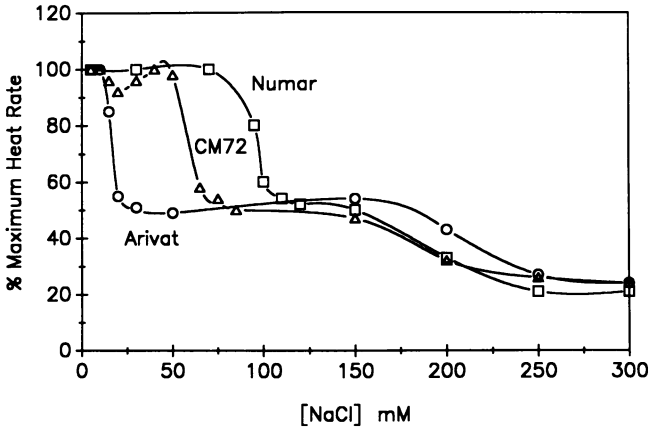

Figure 4. Effects of NaCl concentration on metabolic heat rates of Arivat (○), CM 72 (△), and Numar (□) root tissues. Data for these experiments were all normalized to set rates at low salt to 100% and thus eliminate heat rate differences between samples due only to differing mass of root tissues in each sample. Initial heat rates measured were 208 μW/138 mg for CM 72, 174 μW/116 mg for Arivat, and 285 μW/190 mg for Numar. The experiments were repeated three times. The variation around the midpoints of the downward inflection of the curves was within ±6 mM NaCl as shown in Figure 2.

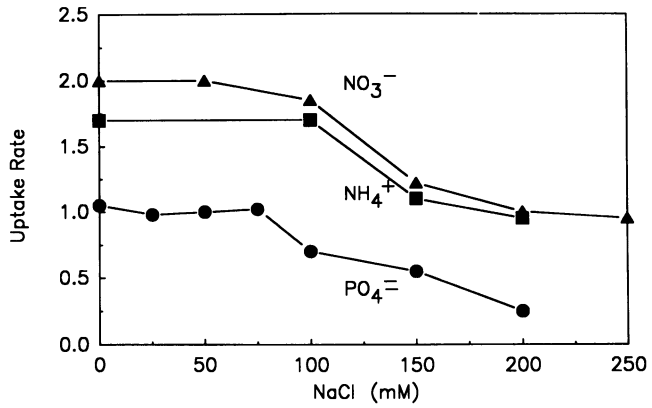

Figure 5. Effect of NaCl on the uptake rates of NO_3^- (△), NH_4^+ (□), and phopshate (○) by CM 72 roots. Rates are expressed as μmol/g tissue/h for NO_3^- and NH_4^+ but as μmol/g/10 h for phosphate. See "Materials and Methods" for procedures.

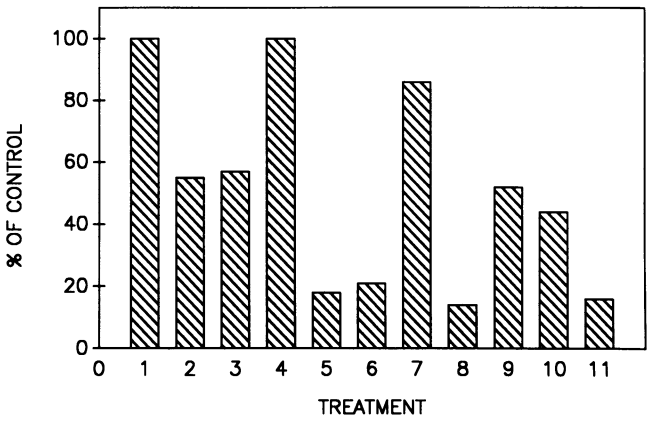

Figure 6. Effects of inhibitors on the metabolic heat rates of barley root tissues. Heat rates of each tissue sample were measured as a control, then potential inhibitors were added and heat rates remeasured. Values are reported as a percentage of control values. Reproducibility of these values was within 5% based on replicate measurements. Heat rates for the control sample in each test exceeded 100 μW. Sample treatments were: 1. control, no treatment; 2. NaCl, 150 mM; 3. NaCl, 150 mM followed by wash with ¼ Hoagland solution to remove salt; 4. mannitol, 300 mM; 5. CN^-, 17 mM; 6. DNP, 2.7 mM; 7. SHAM, 7.5 mM; 8. SHAM + CN^-; 9. Arsenate, 61 μm; 10. NaCl then Arsenate; 11. Arsenate then NaCl.

quarter strength Hoagland nutrient solution. Column 2 once again shows that 150 mM NaCl inhibited about 50% of the metabolic heat rate. Column 3 shows that the NaCl inhibition is not reversible by washing away the salt. Mannitol (300 mM, column 4) had no measurable effect on metabolism, indicating that the decreased metabolic heat rates caused by NaCl were not simply due to osmotic effects. Cyanide (column 5) and DNP[3] (column 6) both caused major reductions in heat rates. The metabolic rates were not reduced to zero by these treatments during the time course of the experiments, but leveled off at about 15 to 20% of the maximum value. SHAM, which inhibits an alternative pathway of oxidation, had only a small effect (column 7). A combination of SHAM and CN^- did not completely block all root tip metabolism (column 8). Arsenate at concentrations producing maximum inhibition decreased metabolic heat output about 50% (column 9). When arsenate was added to roots which had been exposed to 150 mM NaCl, little additional inhibition was noted (column 10). In contrast, when NaCl was added to arsenate inhibited root tissue, a greatly enhanced inhibition was observed (column 11) indicating a dependence of the extent of inhibition upon the order of addition of the salt and arsenate.

[3] Abbreviations: DNP, dinitrophenol; SHAM, salicylhydroxamic acid.

DISCUSSION

These studies have established that metabolic heat rates of root tissues and changes in metabolism induced by salt stress can be readily monitored by isothermal microcalorimetry. Several unexpected results were obtained: (a) the level of salt-sensitive metabolism in barley roots was about 50% of the control, (b) the energy producing reactions inhibited by salt may be linked to the energy utilized in driving nutrient uptake, (c) inhibition of root metabolism by salt is rapid and essentially irreversible over a period of 1 h. In previous experiments (10), it was observed that recovery of NO_3^- uptake following NaCl inhibitor required long-term processes (3–10 h), probably involving protein synthesis.

Below inhibitory concentrations, salt stimulated the metabolic heat rate (about 20%) of roots of seedlings grown in dilute nutrient solution (1/20 strength Hoagland solution) (Fig. 2, top). This result agrees with earlier reports of salt-induced respiration occurring at salt concentrations above 0.5 mM (11, 12, 17). Salt stimulated respiration seems unrelated to injury by NaCl. The concentrations of NaCl causing inhibition of physiological processes such as metabolic heat output (Figs. 1–6), ion uptake (1, 10, 15, 21, 22), growth (21, 22), and possibly membrane damage (10) were significantly higher than concentrations which caused an initial stimulation of respiration.

It was noteworthy that metabolic heat rates were inhibited only about 50% by a salt concentration as high as 200 mM (Fig. 4). Uptake of NO_3^-, NH_4^+, and Pi required somewhat higher salt concentrations for inhibition than did the metabolic heat rate (*cf.* Figs. 4 and 5). The shapes of the response curves and the extent of inhibition were similar, however. Interestingly, the uptake of NO_3^-, NH_4^+, and Pi also reached a new steady state rate about 50% lower at the high salt concentrations (Fig. 5). Intact seedlings were used for uptake curves, whereas detached root tips were used in metabolic heat rate studies. The root tips may be somewhat more sensitive to salt concentrations than intact tissues. Only when salt concentrations were very high (>200 mM) was the less salt-sensitive metabolism blocked. These levels of salt ultimately kill the plants (data not shown).

Several energy requiring steps of metabolism, such as NO_3^- assimilation are maintained at nearly normal ratios (reduction/uptake) in the presence of levels of salt which inhibited uptake and metabolism (1, 10, 21, 22). These assimilatory reactions appear to use metabolic energy pathways different from those used for uptake processes.

Inhibition of metabolic heat output was detected as early as 1 min after treating the roots with salt and was almost

complete after 10 min of treatment (Fig. 3). This is remarkably similar to the time course found for salt-induced inhibition of NO_3^- transport (10). The time required for NO_3^- uptake to recover from salt injury depended upon the length of the salt treatment. The recovery of transport activity appeared to require RNA and protein synthesis and possibly synthesis of new membranes (10). The recovery of uptake required longer time periods (>3 h) than allowed in these studies of metabolic heat output.

The shapes of the curves in Figures 2 and 4, obtained when metabolic heat rates were measured as the roots were titrated with increasing NaCl, indicated abrupt changes over narrow ranges of NaCl concentration. These results fit a commonly postulated model in which increasing salt levels cause general injury to membranes. Evidence which favors a salt-induced injury to plasma membranes includes the displacement of Ca^{2+} and increased efflux of K^+ (2), the loss of ability to discriminate between K^+ and Na^+ (3, 17), the loss of protein from osmotically shocked barley roots which was correlated with decreased Pi uptake (15), and other observations on the ability of Ca^{2+} to partially protect NO_3^- transport from NaCl inhibition (21). Since studies of metabolic heat output indicate that changes in metabolic heat rates and changes in ion transport are closely correlated, membrane damage may provide the link relating these results.

To account for the irreversibility of NaCl inhibition and the high order dependence on $[Na^+]$, a model based on structural change may further postulate that the displacement of Ca^{2+} is linked to some concerted response, such as a general alteration of protein or membrane. The binding of Ca^{2+} to membrane phospholipids is a well documented example of cooperative binding influencing structures (18).

However, the results of these studies are equally compatible with a model in which high salt causes specific inhibition of some reaction (such as an energy production step necessary to drive transport of ions) that would simultaneously affect all the various transport reactions. Lynch and Läuchli (14) recently proposed another model according to which NaCl displaces Ca^{2+} associated with intracellular membranes possibly by depletion of intracellular Ca^{2+} pools and activation of the phosphoinositide system.

Several inhibitors were compared with NaCl as to their effects on metabolic heat output (Fig. 6). Arsenate, like NaCl, blocked only 50% of the metabolism. The pool of heat producing reactions blocked by arsenate was not the same as that blocked by salt, however, since subsequent addition of salt to the arsenate inhibited tissue greatly reduced the measured heat rates. It was of interest to note that when NaCl was added prior to arsenate, the arsenate had apparently no effect on the root metabolism. The reasons for this finding appear quite clear. Arsenate is a metabolic analog of Pi. It was shown in Figure 6 that Pi uptake is inhibited by salt; arsenate uptake may also be inhibited by these conditions. The respiratory inhibitors, cyanide and azide, both blocked about 80 to 85% of metabolic heat output. Dinitrophenol also inhibited about 80% of the heat output. Based on studies with isolated mitochondria, uncoupling with DNP would be expected to increase heat output instead of decreasing it. Jackson *et al.* (9) have shown that the effects of DNP in barley roots are complicated and multifaceted. They recently reported that the effects of DNP on ATP formation in detached barley roots as followed by ^{31}P NMR also showed responses inconsistent with expected uncoupling.

An appreciable level of energy metabolism was found after inhibition with the very strong metabolic inhibitors CN^- and azide (data not shown). This metabolism was not blocked by SHAM. While SHAM did have a small effect upon levels of heat release, combinations of SHAM plus CN^- did not inhibit all metabolism. The residual heat may represent hydrolytic or catabolic processes.

The calorimetric determinations provided quantitative evaluations of the differing sensitivities of three barley cultivars to increasing salt stress (Fig. 4). At concentrations above 150 mM salt, a second level of sensitivity was detected which was not cultivar specific. Our results show that the method provides further understanding of the phenomenon of salt stress and has potential use in initial screening of important crop species for salt tolerance or other stress factors.

LITERATURE CITED

1. **Aslam M, Huffaker RC, Rains DW** (1984) Early effects of salinity on nitrate assimilation in barley seedlings. Plant Physiol **76:** 321–325
2. **Cramer GR, Läuchli A, Polito VP** (1985) Displacement of Ca^{2+} by Na^+ from the plasmalemma of root cells. Plant Physiol **79:** 207–211
3. **Cramer GR, Lynch J, Läuchli A, Epstein E** (1987) Influx of Na^+, K^+, and Ca^{2+} into roots of salt-stressed cotton seedlings. Plant Physiol **83:** 510–516
4. **Criddle RS, Breidenbach RW, Lewis EA, Etough OJ, Hansen LD** (1989) Effects of temperature and oxygen depletion on metabolic rates of tomato and carrot cell cultures and cuttings measured by calorimetry. Plant Cell Environ (in press)
5. **Epstein E, Norlyn JD, Rush DW, Kingsbury RW, Kelley DB, Cunningham GA, Wrona AF** (1980) Saline culture of crops: a genetic approach. Science **210:** 399–404
6. **Goyal S, Huffaker RC** (1986) A novel approach and a fully automated microcomputer-based system to study kinetics of NO_3^-, NO_2^-, and NH_4^+ transport simultaneously by intact wheat seedlings. Plant Cell Environ **9:** 209–215
7. **Goyal S, Huffaker RC** (1986) The uptake of NO_3^-, NO_2^-, and NH_4^+ by wheat (Triticum aestivum) seedlings. I. Induction and kinetics of transport systems. Plant Physiol **82:** 1051–1056
8. **Hoagland DR, Arnon DI** (1950) The water-culture method for growing plants without soil. Calif Agric Exp Stn Cir 347
9. **Jackson PC, Pfeffer PE, Gerasemowicz WV** (1986) Use of ^{31}P NMR to assess effects of DNP on ATP levels *in vivo* in barley roots. Plant Physiol **81:** 1130–1133
10. **Klobus G, Ward MR, Huffaker RC** (1988) Characteristics of injury and recovery of net NO_3^- transport of barley seedlings from treatments of NaCl. Plant Physiol **87:** 878–882
11. **Lundegardh H** (1955) Mechanisms of absorption, transport, accumulation and secretion of ions. Annu Rev Plant Physiol **6:** 1–24
12. **Luttge U, Cram WJ, Laties GG** (1971) The relationship of salt stimulated respiration to localized ion transport in carrot tissue. Z Pflanzenphysiol **64:** 418–426
13. **Luttge U, Pitman MG** (1976) Transport and energy. *In* U Luttge, MG Pitman, eds, Transport in Plants II, Part A, Cells. Encyclopedia of Plant Physiol, Vol 2. Springer-Verlag, Berlin, pp 251–259
14. **Lynch J, Läuchli A** (1988) Salinity affects intracellular calcium in corn root protoplasts. Plant Physiol **87:** 351–356
15. **Mass EY, Ogata G, Finkle MH** (1979) Salt induced inhibition of phosphate transport and release of membrane proteins from barley roots. Plant Physiol **64:** 139–143

16. **Murphy J, Riley JP** (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta **27:** 31–36
17. **Rains DW** (1972) Salt transport by plants in relation to salinity. Annu Rev Plant Physiol **23:** 367–388
18. **Rehfeld SJ, Hansen LD, Lewis EA, Eatough DJ** (1982) Alkaline earth cation binding to large and small bilayer phosphatidylserine vesicles. A calorimetric and potentiometric study. Biochim Biophys Acta **691:** 1–12
19. **Thayer JR, Huffaker RC** (1980) Determination of nitrate and nitrite by high pressure liquid chromatography: comparision with other methods for nitrate determination. Anal Biochem **102:** 110–119
20. **Veen BW** (1980) Energy cost of ion transport. *In* DW Rains, RC Valentine, A Hollaender, eds, Genetic Engineering of Osmoregulation. Plenum Press, New York
21. **Ward MR, Aslam M, Huffaker RC** (1986) Enhancement of nitrate uptake and growth of barley seedlings by calcium under saline conditions. Plant Physiol **80:** 520–524
22. **Ward MR** (1985) Acquisition and assimilation of nitrogen and phosphorus by barley seedlings under saline conditions. MS Thesis, University of California, Davis