Sodium Chloride, Calcium Chloride, and the Respiration of Maize Root Sections

Abstract. The effects of sodium chloride and calcium chloride at concentrations of 0.005*N* and 0.010*N* upon the respiration of vacuolated and nonvacuolated portions of the root tip of maize have been investigated. In both sections $CaCl_2$ produced marked stimulation, while NaCl had no effect. In this tissue stimulation of respiration does not appear to be directly related to metabolic ion accumulation.

It has been shown that the nonvacuolated tissue of the root meristem of *Zea mays* is incapable of actively accumulating Na, Ca, or Cl ions (*1*, *2*). In this tissue active or metabolically mediated accumulation of these ions is observed coincidentally with the appearance of well-defined vacuoles. The nonvacuolated region of the root extends about 1.8 mm from the tip in the variety we used (Peoria). Only beyond this zone is normal active accumulation found.

This finding has enabled us to investigate the effects of salt upon respiration in the absence of metabolic uptake and to compare these effects with those observed in older sections of the same roots in which salt uptake is a function of metabolic activity. So far the effects of NaCl and $CaCl_2$ have been examined.

Maize seedlings were grown in 0.25 $\times 10^{-3}N$ $Ca(NO_3)_2$, and sections of the primary root were cut as described previously (*1*). In this work the sections used were 0 to 1.8 mm and 1.8 to 3.8 mm from the tip, respectively. Oxygen uptake was measured by standard Warburg procedure. In each case respiration was measured at 10-minute intervals in H_2O for a period of 80 minutes, after which the appropriate salt solution was added from the side arm and measurements continued for another 80 minutes. In each experiment the effect of NaCl or $CaCl_2$ upon the respiration of tissue washed in 100 ml of H_2O for 1 hour was compared with the effect upon the respiration of tissue washed in 100 ml of salt at the same concentration to be used subsequently in the Warburg vessel. Forty root segments were used for each experiment. The results are shown in Table 1.

It is apparent from these data that, at the two concentrations tested, NaCl has little if any effect upon the respiratory rate of either section of the root. The second section consists almost entirely of vacuolated tissue and comprises a region of very active metabolic accumulation of both Na and Cl (*1*, *2*). Nevertheless, the effect of NaCl was no greater for this section than for the first section in which only passive uptake could be demonstrated, the two effects being alike almost negligible. Pretreatment with NaCl had no significant effect upon the subsequent respiratory response to this salt of either section.

Calcium chloride at a concentration of 0.005*N* exerted a marked stimulatory effect upon the respiration of both sections. At the higher concentration tested (0.010*N*) the stimulation, while still appreciable, was considerably reduced. Pretreatment with $CaCl_2$ lowered the magnitude of the response in both sections.

In one experiment the effect of pretreating the segments with the chelating agent cyclohexane diamino tetraacetic acid was investigated. This pretreatment drastically lowered the respiration rate in water. Addition of $CaCl_2$ resulted in only a partial recovery (Table 1).

The results of these experiments, we believe, cast considerable further doubt upon theories which relate the salt respiration to the operation of an anion pump (*3*). On the one hand, respiration may be stimulated in the absence of metabolic accumulation ($CaCl_2$, section 1), and on the other hand, active accumulation may occur without significant effect upon the respiration rate (NaCl, section 2). While the results of experiments such as these do not disprove the existence of the cytochrome-mediated mechanism of ion uptake postulated first by Lundegårdh (*4*) and later espoused with modification by Robertson and others (*3*), they do, we believe, illustrate the folly of attempting to prove such existence by the calculation of ratios of anions actively accumulated to oxygen consumed in the salt respiration. In the case of the first segment studied here, all such ratios are perforce meaningless, for no chloride is metabolically absorbed.

The specific effects of cations upon respiration found in our work may well be related to effects upon enzyme systems, upon the physical state of the protoplasm, or perhaps to both. In this connection it is of interest that Hanson (*5*) has demonstrated that Ca protects ribonucleic acid (RNA) from destruction by ribonuclease. He has also found endogenous ribonuclease to be most abundant in the root tip. The effect of Ca upon respiration demonstrated may be due to this protective function. However, Ca affects other enzyme systems, being generally inhibitory to those requiring Mg as a cofactor. The significantly reduced stimulation found when the concentration of Ca was doubled may be due to effects upon these systems. The failure of Na to stimulate respiration is probably to be ascribed to the overriding effect of Ca depletion.

The largest effects of Ca upon respiration were found when the rate in water was lower than average. Although the natural variability of this tissue with respect to respiratory rate prevents any firm conclusions on the basis of data so far obtained, we believe that the initial Ca content of the tissue may well be the limiting factor which determines

Table 1. Effect of NaCl and $CaCl_2$ upon the respiration rate of vacuolated and nonvacuolated tissue of maize roots. Rates are given in microliters per gram per hour, fresh-weight basis; each value is the average of two determinations.

Pretreatment	Medium	Rate (μl/g hr)		Stimulation (%)
		In H_2O	In salt solution	
	Section 1, 0 to 1.8 mm from tip			
H_2O	0.005*N* NaCl	981	1002	2.1
H_2O	0.010*N* NaCl	968	989	2.2
H_2O	0.005*N* $CaCl_2$	851	1050	23.4
H_2O	0.010*N* $CaCl_2$	879	1025	16.6
0.005*N* NaCl	0.005*N* NaCl	1062	1107	4.2
0.010*N* NaCl	0.010*N* NaCl	1078	1118	3.7
0.005*N* $CaCl_2$	0.005*N* $CaCl_2$	1003	1014	1.1
0.010*N* $CaCl_2$	0.010*N* $CaCl_2$	970	993	2.4
0.005*N* CDTA	0.005*N* $CaCl_2$	484	572	18.2
	Section 2, 1.8 to 3.8 mm from tip			
H_2O	0.005*N* NaCl	1155	1117	−3.3
H_2O	0.010*N* NaCl	1052	1065	1.2
H_2O	0.005*N* $CaCl_2$	847	1063	25.5
H_2O	0.010*N* $CaCl_2$	1029	1152	12.0
0.005*N* NaCl	0.005*N* NaCl	910	915	0.5
0.010*N* NaCl	0.010*N* NaCl	1037	1062	2.4
0.005*N* $CaCl_2$	0.005*N* $CaCl_2$	910	1052	15.6
0.010*N* $CaCl_2$	0.010*N* $CaCl_2$	1042	1055	1.2
0.005*N* CDTA	0.005*N* $CaCl_2$	697	848	21.6

the response obtained from addition of Ca and perhaps other ions to the medium.

In previous work (*6*) with whole excised barley roots, $CaCl_2$ was found to have a much smaller effect upon respiration than that found here, while $CaBr_2$ and $CaSO_4$ were entirely without effect. This is probably a reflection of the greater need for Ca of cells close to the root meristem. In the case of excised barley roots the bulk of the tissue was composed of mature cells. There is of course also the possibility that the different responses to Ca found may be due to metabolic differences between the two species. Experiments with maize root sections further from the growing point are expected to illuminate this (*7*).

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References and Notes

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Chlorides Affect the Toxicity of Fluorides to Rainbow Trout

Abstract. Results of an experiment designed to test the effect of chloride ion concentration on fluoride toxicity to rainbow trout *(Salmo gairdnerii)* indicated that tempering fish to chloride reduced their response to fluoride.

On several occasions we have observed that fish collected for fluoride toxicity experiments varied in their group responses to the same concentration of fluoride. In every case these fish came from waters that had different chloride concentrations (*1*). Preliminary investigation indicated that the time required for the top minnow *Gambusia affinis* to succumb to a given concentration of fluoride increased when the normality of chloride was increased. This suggested that the chlorides had an effect on the toxicity of fluorides.

A 2 × 3 × 6 completely randomized factorial experiment, with rainbow trout as subject, was designed as follows: two replications of two qualities of tempering by three concentrations of chloride by six concentrations of fluoride were used. All combinations of these treatments were randomly selected and placed in 72 experimental units. Each unit consisted of five trout ranging from 4 to 7 inches and placed in a 20-gallon aquarium filled with 50 liters of softened water (the calcium and magnesium were reduced in an anion exchange column to a calcium concentration of less than 1 ppm and a magnesium concentration of less than 0.3 ppm). The tempering solutions were of the same quality softened water as the experimental units. The experiment was run for 120 hours at 7°C. All mortality occurred within the first 72 hours.

The fish were first placed into two 300-gallon holding tanks; in one the concentration of chloride (added in the form of sodium chloride) was 34 ppm. In the other it was 0 ppm. The fish were held in these aquaria for 48 hours before they were placed in the experimental tanks. The unit of measurement in the experiment was the number of fish in each unit that responded to the toxin.

Results of the experiment indicated that two factors had very significant effects on the response. The most striking was that tempering to chloride decreased the response of the trout to a given concentration of fluoride. The other was that increasing concentrations of fluoride bring about an increase in fish mortality (Table 1). Table 2 is a statistical analysis of the results.

The LC_{50} (lethal concentration for 50 percent of the experimental subjects) was also found to differ significantly; 6 ppm elicited a response from 50 percent of the nontempered fish, while 22 ppm was required for the tempered fish.

Sensitivities of the fish to fluoride toxication also differed significantly between the tempering treatments. The tempered fish responded less in terms of probits of response per unit increase in concentration of the toxin than the nontempered fish.

A number of authors have alluded to the existence of a specialized chloride secretion mechanism in fish (*2*). Copeland (*3*) states that chloride-secreting cells appear to respond to changes in chloride concentration in the blood. Similarly, we have found an increase in gill epithelium mucous cells when rainbow trout were subjected to increasing concentrations of fluoride (*1*).

The evidence presented by this experiment suggests that the chloride and

Table 1. Number of deaths of rainbow trout in response to combinations of various fluoride and chloride concentrations. The response indicated is the sum mortality in numbers of fish over two replications.

Fluoride concentration (ppm)	Deaths (No.)								
	Fish not tempered				Fish tempered				Total deaths
	At Cl^- concn. indicated			Deaths, subtotal	At Cl^- concn. indicated			Deaths, subtotal	
	0 ppm	3 ppm	9 ppm		0 ppm	3 ppm	9 ppm		
0	2	1	0	3	0	3	0	3	6
2	3	2	0	5	0	0	5	5	10
4	5	4	0	9	0	1	0	1	10
7	5	5	4	14	1	2	0	3	17
13	9	10	6	25	6	5	1	12	37
25	10	10	10	30	10	5	1	16	46
Totals	34	32	20	86	17	16	7	40	126

Table 2. Analysis of the variance of the responses shown in Table 1.

Source	D.F.	Sum of squares	Mean square	F-ratio
Replications	1	8.0000	8.0000	3.077
Tempering	1	29.3889	29.3889	11.303*
Chloride	2	14.2500	7.1250	2.740
Fluoride	5	113.6667	22.7333	8.743*
Tempering × chloride	2	0.3611	0.1805	0.069
Tempering × fluoride	5	16.4444	3.2889	1.265
Chloride × fluoride	10	15.0833	1.5083	0.580
Tempering × chloride × fluoride	10	25.3056	2.5306	0.973
Error	35	91.0000	2.6000	
Total	71	313.5000		

* Significant at the 99-percent level of confidence.