Plant Physiol. (1972) 50, 682–686

Regulation of Salt Respiration in Carrot Root Slices

Received for publication January 17, 1972

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ABSTRACT

In slices of carrot-phloem parenchyma washed for 7 days in water at 20 C, 50 mM KCl stimulates respiration by up to 100% of the ground respiration within 4 minutes of application. The data presented imply that ADP liberated in the cytoplasm as a consequence of KCl accumulation first stimulates a regulator reaction requiring ADP (phosphoglycerate kinase). Thereafter, the point of control alternates between this reaction and the phosphofructokinase reaction, forming a sequence of enzyme stimulations which continue after the new steady state of increased respiration is established. KCl induces a similar sequence in slices washed for 3 days, but it is completed within 3 minutes, and metabolite oscillations are not so marked. In slices washed for 2 days, KCl stimulates respiration by less than 10%, and the sequence of regulator reactions does not occur. Phosphoglycerate kinase is the only enzyme stimulated within 3 minutes of applying KCl to these slices. Contrary to previous reports, KCl frequently stimulates the respiration of freshly prepared slices by 10 to 30%.

The increase in rate of oxygen uptake usually occurring when washed slices of plant tissue are treated with salt solutions was described as "salt" respiration by Robertson (13). Although he initially believed that the extra respiration was essential for accumulation of salt, subsequently Robertson and Thorne (15) observed accumulation without salt respiration, and Atkinson and Polya (5) markedly inhibited respiration of carrot slices with ethionine without seriously inhibiting salt accumulation.

In the experiments reported here, we measured the rate of oxygen uptake immediately after applying KCl to slices of carrot-phloem parenchyma and investigated the reactions regulating the rate of respiration while it was increasing.

As in a previous paper (3), we identify the reactions of glycolysis concerned by applying the crossover theorem of Chance (7), as applied to the glycolytic sequence (10). A site of regulation is identified during a period of increasing influx of respiration as the irreversible reaction at which substrate is depleted and product increased during that period (3, 7). Since 1,3-diphosphoglyceric acid could not be measured in the extracts, the concentration of this compound was assumed to be in quasi-equilibrium with TP[3] (11) and the concentration of the latter used in detecting crossovers.

ADP is a substrate for both PGK and PK and, although one of the two may be the regulator reaction, change in ADP must affect both; since the reactions of the glycolytic series between PGK and PK are reversible, accumulation of 3PGA when PGK is stimulated, or depletion of PEP when PK is stimulated, will tend to be reduced, thus masking crossovers which will then be shown only by depletion of substrate (PGK) or accumulation of product (PK). HK and PFK would be affected in the same way.

The experiments reported here were carried out on slices from the same batches as those used in the previous reports on induced respiration (1, 2, 3).

MATERIALS AND METHODS

Methods used for preparing slices of carrot-phloem parenchyma, for measuring oxygen uptake, and concentrations of metabolites were as previously described (3). Slices were treated with KCl in the electrode flask either by adding concentrated KCl or by changing the liquid in the flask. Washed tissue (60 g) to be treated with KCl was placed in a bag of Fiberglas netting and held in distilled water to equilibrate for 30 min. Excess water was then removed by shaking, and the bag was transferred to 3 liters of 50 mM KCl for the required time, removed again, quickly shaken free of salt solution, and plunged into liquid N_2 for 4 min. A series of extracts using $HClO_4$ (3) was prepared from batches of treated, frozen tissue at 42, 73, and 166 hr (Fig. 1). Most extracts in each series were prepared within the 4 min period in which the respiratory flux changed, for only then does the crossover theorem apply (7, 10). Extracts were also prepared from samples of tissue "treated" with distilled water for up to 5 min as controls for salt treatment. All metabolite concentrations are mean values from at least three analyses on each extract; analyses made on replicate extracts vary by not more than 10% (Table I). Since rate-regulating concentrations of ADP and ATP vary between batches of slices prepared from roots grown from the same batch of seed in the same locality (2), highly reproducible results could not be expected in consecutive experiments and were not attempted.

RESULTS AND DISCUSSION

Salt Respiration in Freshly Sliced Tissue. Although Robertson (13) considered that salt did not stimulate respiration of freshly sliced carrot tissue, experiments using the oxygen elec-

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[3] Abbreviations: HK: hexokinase; PFK: phosphofructokinase; PGK: phosphoglyceratekinase; G1P: glucose-1-P; G6P: glucose-6-P; F6P: fructose-6-P; PEP: phosphoenolpyruvate; PYR: pyruvate; FDP: fructose-1,6-diP; TP: dihydroxyacetone-P + glyceraldehyde-P; 2PGA: 2-P-glycerate; 3PGA: 3-P-glycerate.

trode show that KCl or NaCl between 2 and 100 mM may stimulate respiration by 10% and occasionally by 40% above the ground respiration (Table II). The most common response is an increase of 10 to 15% over the range of 2 to 10 mM KCl, though salt respiration does not occur in all washed slices (15). This variable response to salt is consistent with variation in levels of ADP between batches of carrots (2), those where ADP is low being more prone to show stimulation. In the experiments described in detail, 50 mM KCl induced only a small stimulation in slices washed for less than 40 hr (Fig. 1).

Salt Respiration in Washed Tissue. Figure 1 shows that salt respiration begins to appear at the time of the second stage of induced respiration (3) and reaches a maximum at about 20 hr after the induced peak. The arrows indicate when glycolytic intermediates were extracted from samples of tissue; at 42 hr salt respiration is still slight, but it is distinct at 73 hr. The third set of extracts (166 hr) were prepared after washing for a period typical of many experiments on salt respiration. A trace from the oxygen electrode before and after adding KCl (Fig. 2) shows that the increase in respiration is substantially completed within not more than 4 min; this rapid response occurs in all tissues responding to added salt, irrespective of washing time or KCl concentration. Concentrations of metabolites are constant during a control "treatment" in distilled water.

Tissue Washed 42 Hr. The first stage of the induced respiration is almost complete and no single reaction regulates the rate of respiration of the tissue before salt is applied (3). Stimulation to respiration by added salt is only 9%. Except

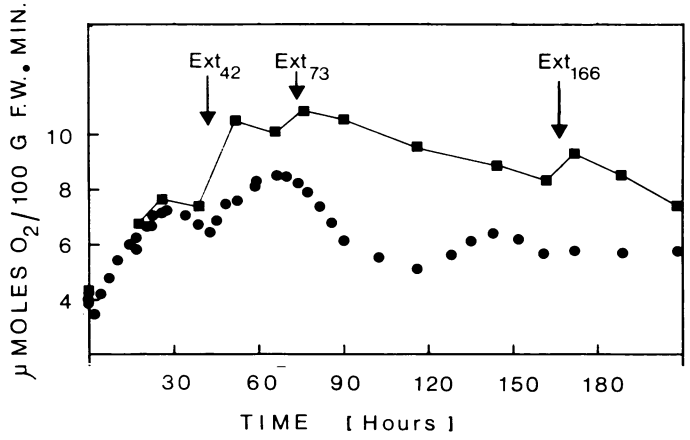

FIG. 1. Effect of 50 mM KCl on the respiration of carrot tissue slices washed for up to 210 hr at 20 C. Labeled arrows show time in hours when extracts (Ext) were prepared for analysis of glycolytic intermediates. ●: Distilled water; ■: 50 mM KCl.

Table II. *Effect of NaCl and KCl on Respiration of Freshly Sliced Phloem Parenchyma prepared from Four Separate Batches of Carrots*

Batch No.	NaCl or KCl	Initial Rate of Respiration	Final Rate of Respiration	Increase
	mM	μmoles O_2/100 g fresh wt·min		%
2	1	4.80	4.80	0
2	2	4.87	5.20	7
3	2	3.37	3.40	1
3	3	3.50	3.61	3
2	4	5.60	6.30	12
3	5	3.68	4.02	9
3	5	5.00	5.40	8
2	10	6.15	7.08	15
3	10	3.68	4.28	16
3	15	3.68	4.72	20
4	15	5.00	6.10	22
2	20	6.19	9.08	47
3	20	4.50	4.95	10
1	50	8.82	10.02	14
1	100	9.57	10.86	14

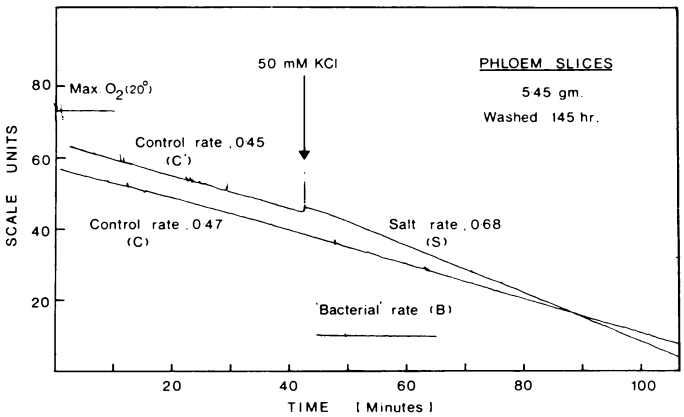

FIG. 2. Traces from the oxygen electrode showing the increase in rate of oxygen uptake in tissue treated with 50 mM KCl (S) above control rates (C). The trace labeled "bacterial rate" (B) shows that the contribution of microorganisms remaining in the medium after the tissue was removed was negligible. Rate of O_2 uptake: scale units per min.

for a crossover at PGK between 2 and 2.5 min (Fig. 3), no other regulator reaction is stimulated while the respiratory flux is increasing. The treatment tends to induce synchronous oscillations in ADP and ATP (Fig. 3).

Tissue Washed 73 hr. The second stage of the induced res-

Table I. *Analyses of Intermediates in Extracts from Replicate Samples of Carrot Tissue*

Metabolite....	Concentration of Intermediates											
	G6P		F6P		PEP		PYR		FDP	TP	ADP	ATP
Batch.....	2	3	2	3	1	3	1	2	2	2	4	1
	μmoles per 100 g fresh weight											
Sample 1	13.5	21.1	1.8	4.42	6.80	1.35	8.55	2.1	1.4	0.85	13.0	30.0
2	13.5	20.8	1.9	4.34	6.67	1.21	8.56	2.2	1.6	0.75	13.5	29.4
3	13.0	21.5	1.9	4.30	5.80	1.27	9.09	2.3	1.5	0.75	13.4	31.8
4	13.2		2.0		6.40	1.46	8.80	2.2	1.45	0.81	13.9	31.8

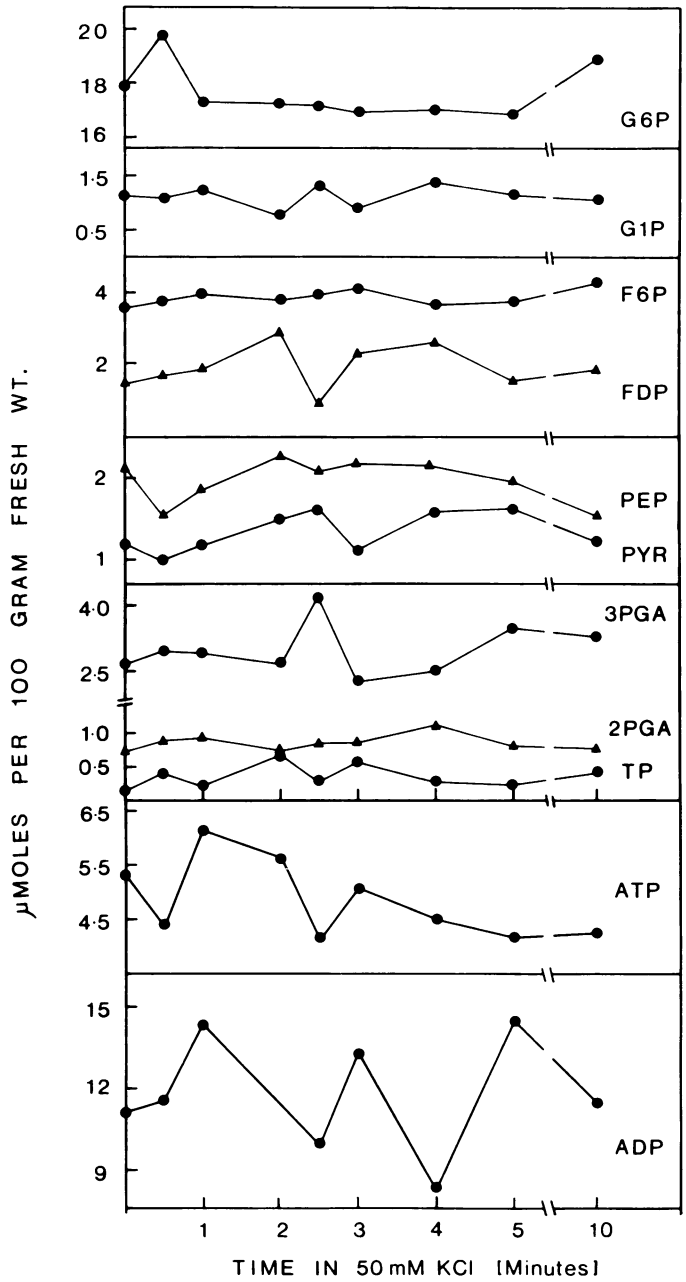

FIG. 3. Changes in concentration of glycolytic intermediates and adenosine pyrophosphates induced by 50 mM KCl in carrot slices washed for 42 hr.

piration is now declining, and as PGK (-ve crossover) regulates glycolysis (3), ADP concentration is the major factor controlling respiratory rate in the untreated tissue. Salt respiration is 33% greater than the control value and crossovers are induced at PGK between 0 and 0.5 and between 2 and 2.5 min, followed by one at PFK between 2.5 and 3 min (Fig. 4). An initial ADP rise, apparently initiating the first PGK crossover, is followed by synchronous oscillations in ADP and ATP (Fig. 4) until 3 min after treatment. The ATP concentration decreases to approximately half the initial value at 4 min, then rises again after the increase in respiratory flux is completed.

Tissue Washed 166 Hr. Respiration rate of untreated 166-hr slices is steady and there are no crossovers at kinase enzymes. KCl stimulates respiration by 50%. PGK and PFK are stimulated alternately during the first 2.5 min, showing more distinct and frequent crossovers than in tissue washed for 42 or 73 hr. ADP and FDP oscillate out of phase between 0.5 and 4 min, the increase in ADP coinciding with stimulation of PGK for the first two cycles. Pyruvate accumulates 100-fold between 4 and 6.5 min but is then depleted. ATP decreases to 4 min as with 73 hr treatment and ADP oscillates with a larger amplitude than at 73 hr (Fig. 5). Atkinson *et al.* (4) have also reported a decrease in ATP below control level in well washed slices treated with KCl. A preliminary experiment with 166-hr tissue showed similar changes to those illustrated in Figure 5. In both 73- and 166-hr slices, salt stimulates PGK and PFK before a new steady state of increased respiration is attained and before ATP level drops to a low value.

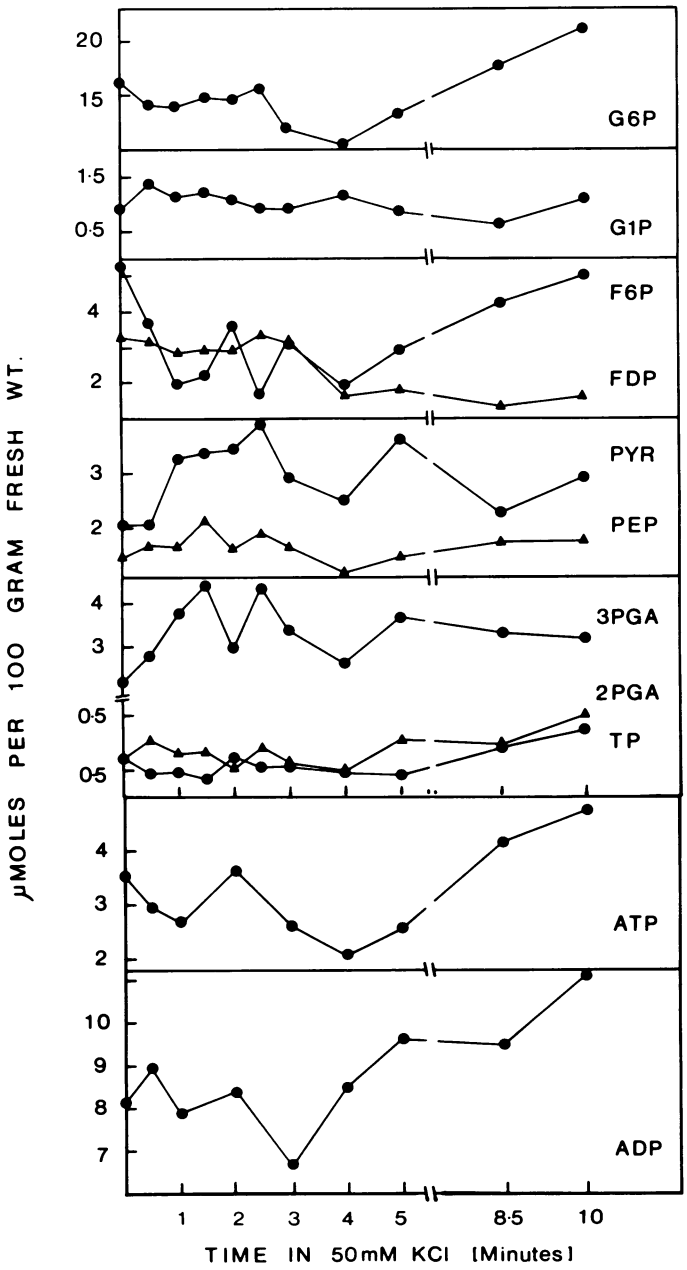

FIG. 4. Changes in concentration of glycolytic intermediates and adenosine pyrophosphates induced by 50 mM KCl in carrot slices washed for 73 hr.

The experiments described above indicate that treating well washed slices with KCl rapidly increases total ADP within 30 sec (Fig. 5). Since a crossover occurs during this time at PGK (Fig. 6), we conclude that much of the increase of ADP occurs in the cytoplasm. Increased cytoplasmic ADP could result from either direct utilization of ATP in salt accumulation as proposed by Sutcliffe and Hackett (17), or by slowing of oxidative phosphorylation when accumulation is coupled with charge separation, as proposed in the chemiosmotic hypothesis (4, 12, 14). Our experiments do not distinguish between these two alternative mechanisms.

The observation that ADP increases in tissue treated with salt is in accord with the marked stimulation of respiration upon adding ADP to well washed slices (1). The increase in formation of ATP following this crossover at PGK in turn appears to stimulate PFK, thus initiating a series of crossovers alternating between PGK and PFK (Table III) within the first 3 min of salt treatment as each reaction tends to overshoot (Fig. 6). A causal relationship between increased turnover of glycolytic enzymes initiated by increase in ADP and salt respiration seems clear.

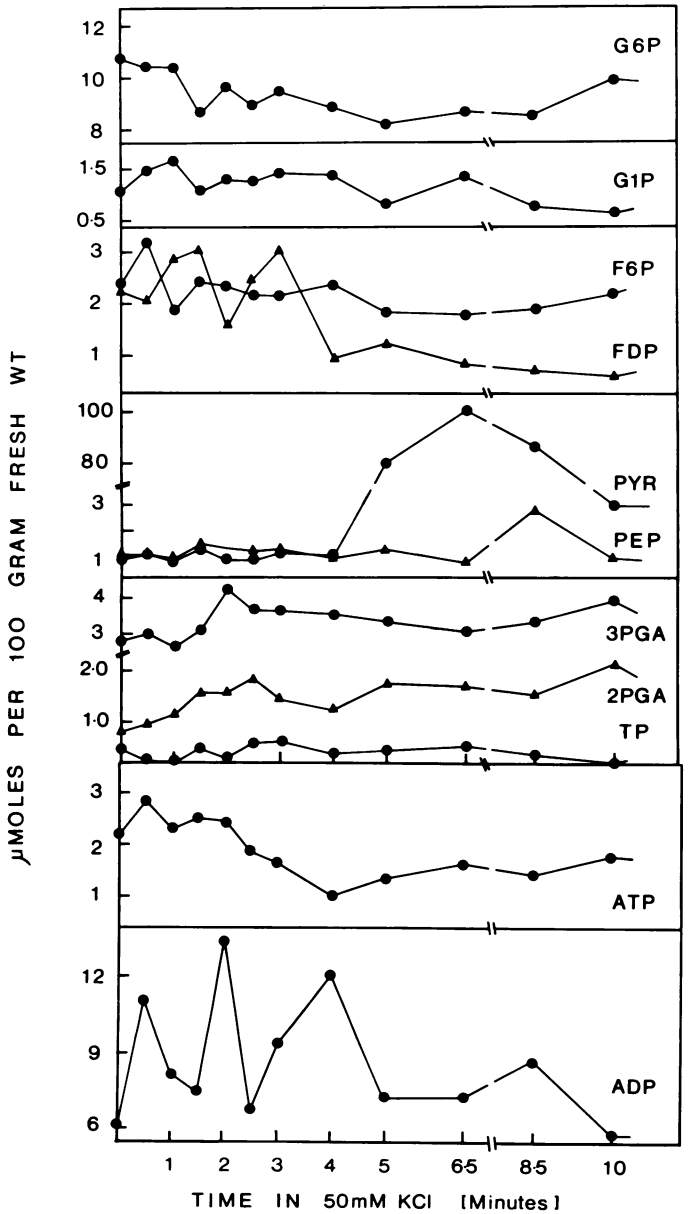

FIG. 5. Changes in concentration of glycolytic intermediates and adenosine pyrophosphates induced by 50 mM KCl in carrot slices washed for 166 hr.

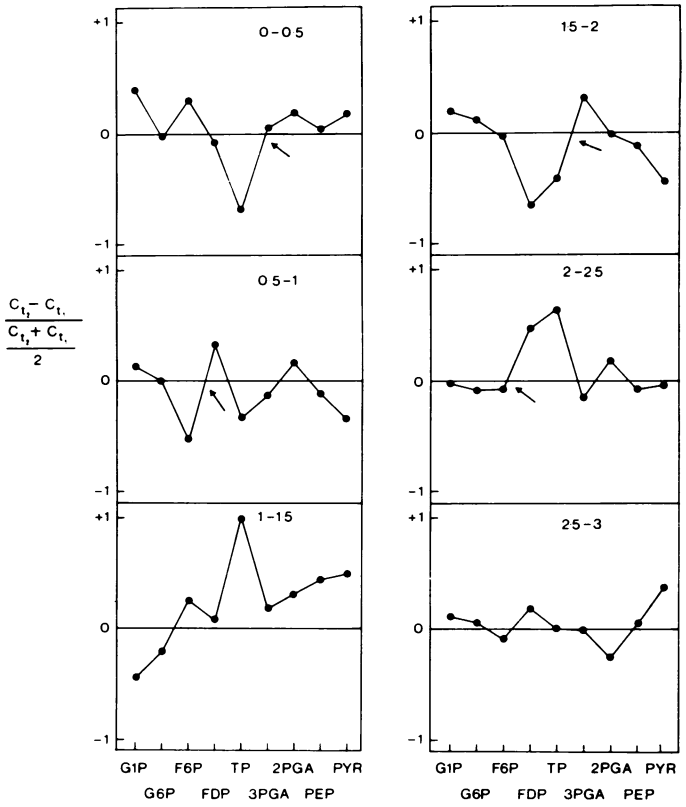

FIG. 6. Crossover diagrams for glycolytic intermediates during the first 3 min after treating carrot slices with 50 mM KCl. C_{t_1} and C_{t_2} are concentration of metabolites measured at consecutive half-minute intervals. The time interval (min) between analyses is shown in figures above each diagram. Slices washed 166 hr. Data are from Figure 5. Arrows indicate major crossovers.

Table III. *Sequence of Enzyme Stimulation deduced from Crossovers during the First 4 Min after Treating Slices Washed for 42, 73, and 166 Hr with 50 mM KCl*

Period in 50 mM KCl	Position of Crossovers		
	42 hr	73 hr	166 hr
min			
0–0.5		PGK	PGK
0.5–1.0		PK	PFK
1.0–1.5		PGK	
1.5–2.0			PGK
2.0–2.5	PGK	PGK	PFK
2.5–3.0		PFK	PFK
3.0–4.0	PGK		

The abrupt increase in pyruvate in tissue washed 166 hr suggests that pyruvate dehydrogenase has become saturated towards the end of the increase in respiratory flux (Fig. 5), and that the pyruvate is gradually depleted when the respiration rate adjusts to the new steady state. Activity of pyruvate dehydrogenase may be low, as observed in potato-tuber slices (8).

Since ADP is a negative effector of PFK in carrot tissue

(9), the out-of-phase oscillations of FDP and ADP could reflect allosteric regulation and over-correction of PFK in the manner described in yeast (10). The concentration of ADP in the slices is high (Fig. 5) and need only be restricted to 10% of total cell volume to act as a negative effector, since it is effective as an inhibitor at 1 mM and above (3, 9).

A small or negligible salt respiration in freshly sliced tissue is consistent with the hypothesis that slicing *per se* stimulates respiration through increasing cytoplasmic ADP (3), thus making further stimulation by salt-induced increase in ADP ineffective. Consequently, salt respiration only begins to develop during the second stage of the induced respiration (Fig. 1), when ADP is falling to a rate-limiting level (3). The high concentrations of ADP occasionally found in some batches of slices (2) would account for a reported absence of a typical salt respiration in some well washed carrot tissue (15).

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