EFFECTS OF L-ETHIONINE ON ADENOSINE TRIPHOSPHATE LEVELS, RESPIRATION, AND SALT ACCUMULATION IN CARROT XYLEM TISSUE

By M. R. Atkinson* and G. M. Polya*

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Summary

The time course during aging of carrot xylem disks for development of salt respiration and sensitivity of respiration to inhibition by L-ethionine was determined. 5 mM L-ethionine has no effect on the respiration of freshly cut disks, but sensitivity developed during the aging process and was maximal from 25 to 120 hr, falling to zero at 150 hr. Maximal inhibition (35–40%) coincided with the period of maximum basal respiratory rate.

Rb^+ uptake into carrot xylem disks aged for 77 hr was linear and at 80% of the control rate for nearly 2 hr in the presence of 5 mM L-ethionine, despite inhibition of salt respiration and a concomitant drop in the ATP level to 50% of control values during this period.

5 mM L-ethionine failed to inhibit Rb^+ and Cl^- uptake into disks aged for 125 hr, although the ATP level fell to 40% of control after 2 hr and both basal and salt respiration were inhibited; 90% of the salt respiration was eliminated within 60 min. Cl^- uptake from 40 mM NaCl into disks aged for 125 hr proceeded linearly at 85% of the control rate for over 2 hr despite a 60% drop in ATP level.

A lag in the onset of oligomycin inhibition of K^+ and Cl^- uptake into aged carrot disks and of KCl uptake into aged beetroot disks was observed. Anaerobic conditions strongly inhibited Cl^- uptake into aged carrot disks over 30 min, although the ATP level fell by only 30%.

The results provide further evidence that salt accumulation into aged carrot xylem tissue is linked to electron transport rather than to hydrolysis of ATP, and indicate that a large proportion of salt respiration may be an indirect consequence of salt accumulation.

I. Introduction

Accumulation of salts by aged slices of plant storage organs is associated with increased respiration, the increase being defined as "salt respiration" (for reviews see Robertson 1960; Briggs, Hope, and Robertson 1961). Salt accumulation is inhibited by anaerobic conditions or by inhibitors of the coupling between mitochondrial electron flow and associated energy-requiring processes. In carrot slices this inhibition often precedes marked decreases in ATP levels, and it has been proposed (Atkinson *et al.* 1966) that there is a direct coupling between salt uptake and electron flow, and no evidence for an involvement of ATP of the type proposed in models of salt uptake involving the Na^+–K^+ adenosine triphosphatases of animal cell membranes (for reviews see Skou 1965; Albers 1967; Heinz 1967). Though a number of salt-stimulated adenosine triphosphatases of plants have been described,

* School of Biological Sciences, Flinders University of South Australia, Bedford Park, S.A. 5042.

there is no evidence that these are involved in ion transport through membranes (Atkinson and Polya 1967).

This paper describes attempts to obtain further information about mechanisms of salt accumulation in plants by lowering the level of ATP with a trapping system. L-Ethionine decreases the ATP content of animal tissues by forming *S*-adenosyl ethionine through the action of *S*-adenosyltransferase (EC 2.4.2.13) (Slater and Sawyer 1966) and studies by Davies (1961, 1964, 1966) on the effects of L-methionine indicate the existence of this enzyme in aged slices of plant storage organs.

It is shown here that ethionine causes a rapid decrease in the ATP content of carrot slices that have been aged for an appropriate time, and that this decrease is associated with a marked inhibition of basal and salt respiration during a period in which there is little or no inhibition of salt uptake. The results are discussed in relation to recent work on the thermodynamics of ion transport into mitochondria, and are shown to provide further support for the direct dependence of salt accumulation on oxidation–reduction systems (Lundegardh 1955, 1960; Robertson 1960; Atkinson *et al.* 1966).

II. Methods

Preparation of Disks

Disks (1 by 10 mm) were cut from xylem parenchyma of carrot (*Daucus carota* L.), commercially obtained from the same source for all experiments except those involving oligomycin. The disks were aged at 21–22°C in distilled water containing 50 mg chloramphenicol per litre. The disks were aerated with filtered air, and the washing solution was changed daily. Parenchyma slices (1 by 5 mm) from commercially obtained beet (*Beta vulgaris* L.) were prepared as above. The disks were aged for 7 days at 25°C, with daily changes of distilled water.

Manometry

Respiratory rates of carrot disks were measured by standard manometric techniques with air as the gas phase and a shaking rate of 120/min (Umbreit, Burris, and Stauffer 1964).

ATP Determination

For each assay, 15 disks were threaded on nylon with 1-mm polythene tubing spacers and washed for 9–16 hr before the start of an experiment. The set of disks (0·60–1·40 g) was frozen in liquid nitrogen and disrupted in 10 ml ice-cold 0·4N perchloric acid–1·0 mM EDTA (disodium salt) with an Ultra-Turrax blender (Janke and Kunkel KG., Staufen, West Germany). This suspension was centrifuged at 10,000 *g* for 15 min; the supernatant was filtered through muslin and brought to about pH 7·4 with 1M KOH–0·1M $KHCO_3$. Potassium perchlorate was filtered off before nucleotide assay. The ATP content of extracts was measured in a scintillation counter with luciferin–luciferase using internal standards as described by Atkinson *et al.* (1966).

ADP Determination

To 5 ml neutralized carrot extract (prepared as for ATP determination) was added 0·25 ml 0·1M $MgCl_2$, 0·01 ml 100 mM phosphoenolpyruvate, and 10 μl (0·32 mg) pyruvate kinase. After mixing and incubation at 20°C for 20 min, a treatment that converts at least 99% of the ADP present into ATP, each extract was assayed for ATP by the luciferin–luciferase method to give an estimate of ADP+ATP initially present in the extract. Subtraction of the value for the ATP in the extract gave the ADP content.

Ion Uptake

Disks (10–15 g, in sets weighing 1·5–2·5 g) were threaded and washed for 16 hr before tracer experiments and were then aerated at 30°C in 200 ml 40 mM RbCl (10–25 μc ^{86}Rb per litre) or 40 mM NaCl (40 μc ^{36}Cl per litre). Samples were removed at appropriate intervals up to 3 hr,

rinsed in 40 mM KCl or NaCl at room temperature (15 sec in each of four separate solutions), and aerated in distilled water at 0–2°C for 30 min to remove radioactive ions from the free-space. Disks were blotted dry, weighed, and subjected to two 30-min extractions into distilled water at 100°C. Uptake of ^{86}Rb and ^{36}Cl was determined using a low-background, gas-flow counter (Model 4342, Nuclear Chicago, Des Plaines, Illinois).

In experiments involving oligomycin, the inhibitor (50 μl of 0·6% ethanolic solution) was added to the external solutions (25 ml) after a 30-min period for free-space equilibration. Batches of disks (5 g/25 ml external solution) were aerated with filtered air at 25°C. Disks were sampled at appropriate intervals and were aerated at 1°C for two 10-min intervals, to remove salt from the free space, and then extracted with boiling water.

K^+ and Na^+ were determined with a flame photometer (EEL, Halstead, Essex, England); Cl^- was determined with a chloride electrode or by electrometric titration.

Conductivity measurements were carried out in a conductivity cell using a Pye conductance bridge as described by Robertson and Turner (1945).

Chemicals

Disodium adenosine triphosphate, firefly tails, phosphoenolpyruvate, pyruvate kinase, and L-ethionine were obtained from Sigma Chemical Co., St. Louis, U.S.A. $^{86}RbCl$ and $Na^{36}Cl$ were obtained from the Australian Atomic Energy Commission, Lucas Heights, N.S.W. Oligomycin used in this work was generously provided by Professor E. McCoy. The oligomycin was chromatographed on Silica Gel-G (E. Merck and Co.) in chloroform–methanol (49 : 1, v/v). The chromatogram was dried and solvent was re-run through the same silica layer to increase resolution before elution with ethanol and spectrophotometric assay. The oligomycin used contained a single component with R_F 0·33 and λ_{max} 225 and 233 mμ. Spectroscopic alcohol (British Drug Houses Ltd., Poole, Dorset) was used to prepare stock solutions of oligomycin.

III. Results

(*a*) *Inhibition of Respiration of Carrot Xylem Disks by* L-*Ethionine*

Methionine inhibits respiration of plant storage tissue that has been aged after cutting (Davies 1961), and it was necessary to find a suitable aging period that would give slices that combined salt uptake and salt respiration with response to ethionine. Changes of respiration, salt respiration, and respiratory sensitivity to ethionine during aging are shown in Figure 1(*a*). Salt respiration was observed after 70 hr of washing. 5 mM L-ethionine had little effect on the respiration of freshly cut carrot disk (washed for 4 hr), but respiratory sensitivity to ethionine developed within 30 hr and was maintained up to 120 hr. After 155 hr of washing the respiration again became insensitive to inhibition by ethionine.

Figure 2 shows the percentage respiratory inhibition by 5 mM L-ethionine, measured with and without 40 mM KCl, as a function of time of aging. When measured at 37°C, basal respiration and respiration in the presence of salt were inhibited to a similar extent; in experiments at 30°C in the presence of RbCl a greater proportional decrease of salt respiration was observed [cf. Figs. 3(*b*), 4(*c*)]. The period of maximal inhibition by ethionine (30–120 hr of washing) coincides with a period of increased respiratory rate.

The temperature optimum for inhibition of respiration by ethionine was found to be near 30°C in slices aged for 78 hr; at 25, 37, and 40°C inhibition was 48, 78, and 14% of this maximum value. At 37°C the respiratory rates, in water or in 40 mM KCl, of slices that had been washed for 119 hr were inhibited to the same extent

in the period from 35–120 min after addition of ethionine [Fig. 1(*b*)]. During this period the inhibition increased from 15–20% to 42–43%. Whereas the experiments

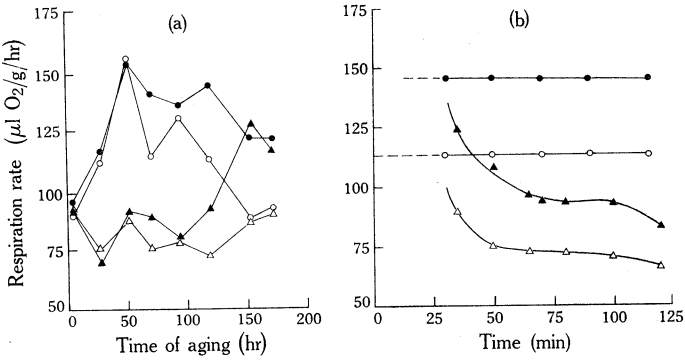

Fig. 1.—(*a*) Time course of development of salt respiration and sensitivity of respiration to L-ethionine in carrot xylem disks which had been aged in water at 22°C for the periods indicated. Disks were blotted gently, transferred to the solutions indicated, and respiration rate measured after incubation for 90 min at 37°C. ○ Water. ● 40 mM KCl. △ 5 mM L-ethionine. ▲ 5 mM L-ethionine+40 mM KCl. (*b*) Time course of inhibition by L-ethionine of the respiration of carrot xylem disks aged for 119 hr at 22°C, and incubated in the solutions indicated from zero time. Treatment of disks and symbols used on figure as in (*a*). In (*a*) and (*b*), respiratory rates were determined from lines of best fit to the manometric data.

in Figure 1(*a*) (to define optimum aging times) were carried out at 37°C, where Davies (1964) had found a sharp optimum for the L-methionine effect, all other experiments were carried out at 30°C to permit comparison with other studies of salt uptake.

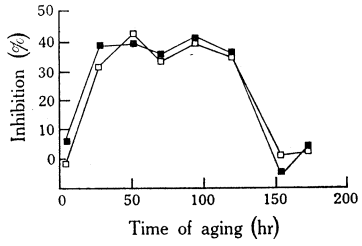

Fig. 2.—Percentage inhibition of respiration in carrot xylem disks aged in water at 22°C for the periods indicated in the absence (□) and presence (■) of 40 mM KCl. Inhibition determined from respiratory rates [see Fig. 1(*a*)] after incubation for 90 min at 37°C.

(*b*) *Effect of* L-*Ethionine on Salt Accumulation, ATP Level, and Respiration in Aged Carrot Xylem Slices*

Net Rb^+ uptake from 40 mM RbCl into carrot xylem slices aged for 77 hr measured with ^{86}Rb is shown in Figure 3(*a*). With no ethionine present, Rb^+ was taken up at the rate of 3·1 μ-equiv/g fresh weight/hr at 30°C. With 5 mM L-ethionine present, uptake was linear for nearly 2 hr at 80% of the control rate (2·5 μ-equiv/g fresh weight/hr); uptake was severely inhibited after 2 hr. Respiration after 20 min incubation with 5 mM L-ethionine [Fig. 3(*b*)] was 21% greater in the presence of salt than without added salt but salt respiration was eliminated after 2 hr of treatment, at which time Rb^+ uptake had ceased. Oxygen uptake rates of disks not treated with

L-ethionine were constant over this period for disks incubated in water or 40 mM RbCl. Figure 4(*a*) shows the fall in ATP level in these disks on treatment with L-ethionine. Although the disks took up Rb^+ linearly for nearly 2 hr at 80% of the control rate, the ATP level dropped by 50% over this period. After 3 hr of treatment with ethionine, the ATP level had fallen to less than 30% of control levels. The larger standard deviations at the 1-hr sampling, and the recovery in the control between 2 and 3 hr may be transient responses to handling and transfer of samples.

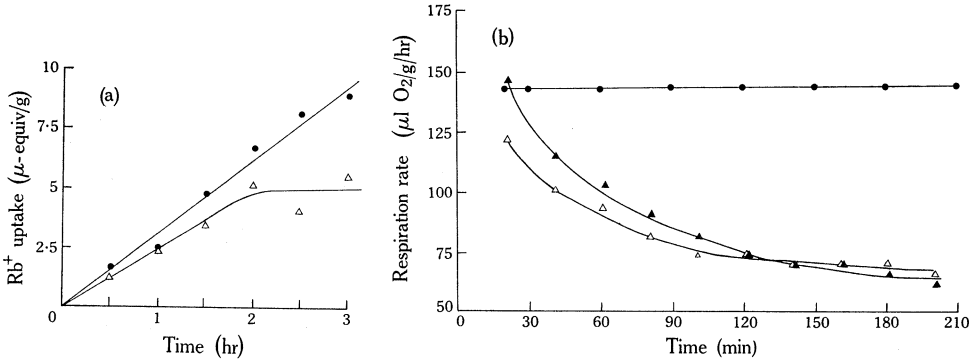

Fig. 3.—(*a*) Effect of L-ethionine on Rb^+ uptake by carrot xylem disks aged for 77 hr. Rb^+ uptake at 30°C from 40 mM RbCl (●) and 5 mM L-ethionine+40 mM RbCl (△) was calculated from net $^{86}Rb^+$ uptake. (*b*) Effect of L-ethionine on respiration of carrot xylem disks aged in water at 22°C for 77 hr and incubated in the solutions indicated from zero time. ● 40 mM RbCl. △ 5 mM L-ethionine. ▲ 5 mM L-ethionine+40 mM RbCl.

Figures 4(*b*) and 4(*c*) show the response to 5 mM L-ethionine of carrot xylem disks aged for 125 hr and incubated in 40 mM RbCl. Uptake of ^{86}Rb by the disks was unaffected by L-ethionine over 2 hr; uptake rates were 4·1 μ-equiv. Rb^+/g fresh weight/hr (without L-ethionine) and 3·8 μ-equiv. Rb^+/g fresh weight/hr (with L-ethionine). Similarly, net chloride uptake was unaffected by L-ethionine over 2 hr, Cl^- uptake rates being 3·6 μ-equiv/g fresh weight/hr (without L-ethionine) and 3·8 μ-equiv/g fresh weight/hr (with L-ethionine added). Over the same period, the ATP level in ethionine-treated disks fell to 40% of the control level [Fig. 4(*b*)]. Figure 4(*c*) shows the respiratory response to L-ethionine of disks aged for 125 hr. Disks incubated without inhibitor showed 41% salt respiration, rates of oxygen consumption being 144 μl O_2/g fresh weight/hr in 40 mM RbCl and 102 μl O_2/g fresh weight/hr in water. L-Ethionine eliminated over 90% of the salt respiration within 60 min at 30°C. From 60 to 120 min, during which interval Cl^- and Rb^+ uptake continued undiminished at 3·6–3·8 μ-equiv/g fresh weight/hr, the salt respiration was only 3–4 μl O_2/g fresh weight/hr (0·3–0·4 μ-atoms oxygen/g fresh weight/hr).

In a separate experiment Cl^- uptake by carrot disks, aged for 125 hr, from 40 mM NaCl at 30°C, measured with ^{36}Cl, was only slightly inhibited by 5 mM L-ethionine [Fig. 5(*a*)]. Over 2·25 hr, disks treated with ethionine took up Cl^- linearly at the rate of 4·7 μ-equiv. Cl^-/g fresh weight/hr, 84% of the rate of uptake with untreated disks (5·6 μ-equiv. Cl^-/g fresh weight/hr). The rate of Na^+ uptake in the control over 3 hr was 5·3 μ-equiv/g fresh weight/hr, a rate close to the Cl^-

uptake rate determined with ^{36}Cl. After 2·5 hr of incubation with ethionine the rate of Cl^- uptake fell to about 30% of the control. In the presence of L-ethionine the ATP level of the slices decreased continuously to 35% of the control value during 2 hr [Fig. 5(*c*)] but Cl^- uptake continued linearly at 84% of the control rate over this period. After 3 hr of treatment with L-ethionine, the ATP level of the disks was about 25% of the control value.

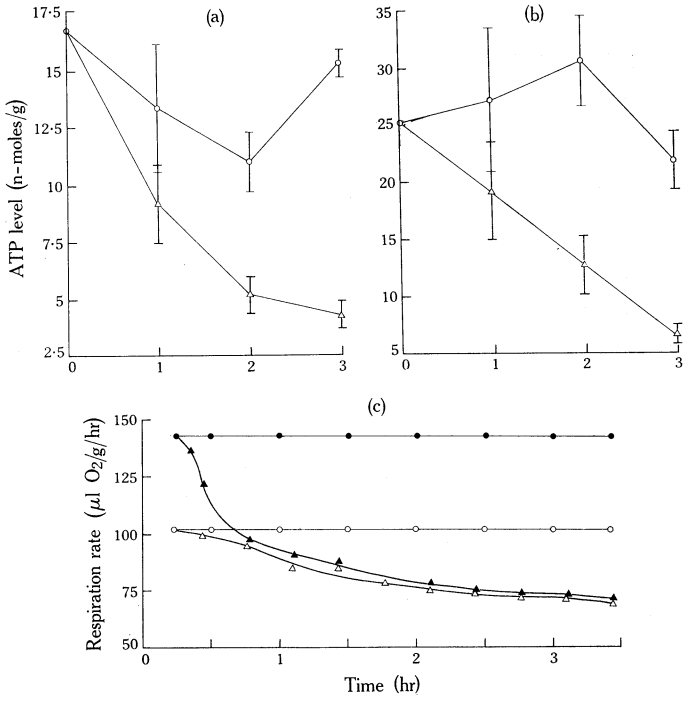

Fig. 4.—(*a*),(*b*) Effect of L-ethionine on the ATP level in carrot xylem disks aged in water at 22°C for 77 hr (*a*) and 125 hr (*b*), and incubated at 30°C in 40 mM RbCl (○) and 5 mM L-ethionine+40 mM RbCl (△). ATP levels were determined in triplicate using disks from the same batch used in ion uptake and respiration experiments. Vertical bars represent standard deviations. (*c*) Effect of L-ethionine on the respiration of carrot xylem disks aged in water at 22°C for 125 hr, and incubated from zero time at 30°C in water (○), 40 mM RbCl (●), 5 mM L-ethionine (△), and 5 mM L-ethionine+ 40 mM RbCl (▲). Respiratory rates were determined from lines of best fit to the manometric data.

(*c*) *Effect of Anaerobic Conditions on Cl^- Uptake and ATP Level in Aged Carrot Xylem Disks*

Figure 5(*b*) shows the effect of anaerobic conditions on uptake of Cl^-, measured with ^{36}Cl, into carrot disks aged for 130 hr. Imposition of anaerobic conditions caused an immediate decrease of Cl^- uptake; Cl^- uptake was largely inhibited after 30 min. Over the same period, the ATP level in the tissue dropped by only 30% [Fig. 5(*d*)]. This result confirms the observation by Aktinson *et al.* (1966), based on

conductivity measurements, that anaerobic conditions quickly inhibited KCl accumulation.

Table 1 shows that in anaerobic conditions, the drop in ATP level in carrot xylem disks aged for 8 days is associated with a rise in ADP level. Attempts to

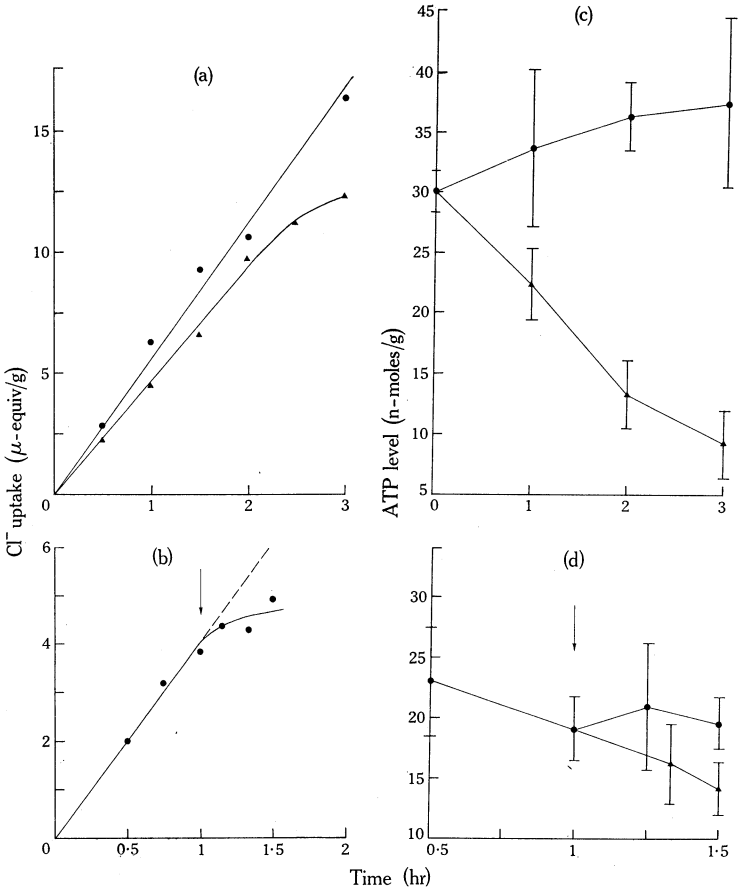

Fig. 5.—(*a*) Effect of L-ethionine on Cl^- uptake at 30°C into carrot xylem disks from 40 mM NaCl (●) and 5 mM L-ethionine + 40 mM NaCl (▲). Disks aged in water at 22°C for 125 hr. (*b*) Effect of anaerobic conditions (arrow) on Cl^- uptake at 30°C from 40 mM NaCl into carrot xylem disks aged in water at 22°C for 133 hr. In (*a*) and (*b*) Cl^- uptake determined from net ^{36}Cl uptake. (*c*) Effect of L-ethionine on ATP levels in carrot xylem disks aged in water at 22°C for 125 hr and incubated at 30°C in 40 mM NaCl (●) and 5 mM L-ethionine + 40 mM NaCl (▲). ATP levels were determined in triplicate using disks from the same batch used in the Cl^- uptake experiment. (*d*) Effect of anaerobic conditions (arrow) on ATP levels in carrot xylem disks aged in water at 22°C for 133 hr and incubated in 40 mM NaCl at 30°C from zero time. Nitrogen bubbled through the test solution after 1 hr. ● Aerobic conditions. ▲ Anaerobic conditions. In (*c*) and (*d*) vertical bars represent standard deviations.

measure AMP by adding adenylate kinase as well as pyruvate kinase in the coupled assay system failed, probably because of the high Michaelis constant (*c.* 10^{-4}M AMP)

which results in very slow phosphorylation of AMP when it is present at about 10^{-6}M in assay mixtures. Net decreases of ATP+ADP (Table 1) may result from conversion of ADP into AMP through the action of adenylate kinase.

TABLE 1

ATP AND ADP LEVELS IN AGED CARROT XYLEM DISKS IN AEROBIC AND ANAEROBIC CONDITIONS

Disks were aged for 7 days and aerated 1 day further for recovery after threading on nylon. Before sampling and extraction disks were aerated for 30 min in 40 mM KCl (treatments A and B) or water (treatments C and D) at 22°C, and then either aerated for a further 80 min (treatments A and C) or made anaerobic by bubbling oxygen-free nitrogen for 80 min (treatments B and D). Values are in n-moles/g of tissue slices. Standard deviations are given from triplicate estimations

Treatment	ATP	ADP	ADP/ATP
A	31±5	6±5	0·19
B	13±1	10±3	0·77
C	28±3	4±1	0·14
D	11±1	14±2	1·27

(d) Effect of Oligomycin on K^+ and Cl^- Uptake in Aged Carrot and Beet Root Slices

Atkinson *et al.* (1966) have shown that KCl accumulation from 40 mM KCl by aged carrot disks, as measured by conductivity, was unaffected for as long as 1 hr in the presence of oligomycin, an inhibitor which blocks phosphorylation of ADP (Lardy, Johnson, and McMurray 1958), but not the uptake of cations linked to electron transport in plant and animal mitochondria (Millard, Wiskich, and Robertson 1964; Rottenberg and Solomon 1966; Goh and Wiskich 1967).

In view of the inhibition of K^+ and Cl^- transport into oat roots by oligomycin (Hodges 1966), it was of interest to re-examine the effect of oligomycin on uptake of K^+ and Cl^-, measured separately rather than by conductivity, into aged carrot disks.

Figure 6 shows that both net K^+ uptake and net Cl^- uptake into aged carrot xylem disks were not inhibited by 12 μg oligomycin/ml (150 μg/g fresh weight of slices) for 1 hr; however, net uptake of both ions was strongly inhibited in the period from 2 to 4 hr after exposure to oligomycin.

As an indication of the variation between different tissues in sensitivity to oligomycin, Figure 7 shows the much more rapid onset of inhibition of KCl uptake by oligomycin in aged beet slices. Uptake was not inhibited for 15 min, but after this period uptake rates diminished until net KCl uptake ceased after 3·5 hr. In another experiment, net K^+ and net Cl^- uptake was completely inhibited by oligomycin (6·7 μg/ml; 20 μg/g fresh weight) after 2 hr of treatment; net loss of both K^+ and Cl^- occurred in the period 2–4 hr after addition of oligomycin.

IV. Discussion

The inhibition of respiration in aged turnip root slices by methionine is associated with an accumulation of *S*-adenosyl methionine through the action of ATP: L-methionine *S*-adenosyl transferase (Davies 1966); and ethionine resembles methionine by acting as a substrate for this enzyme (Fowden, Lewis, and Tristram 1967). If this is the basis of the inhibition of respiration by L-ethionine, then the results shown in Figure 1(*a*) indicate that this enzyme is formed or becomes functional after slicing of the fresh carrot xylem. The ethionine trap may be contrasted to the 2-deoxyglucose–hexokinase trap, which decreases the phosphorylation level of the AMP–ADP–ATP system without directly decreasing the total concentration of adenine nucleotide (McComb and Yushok 1964).

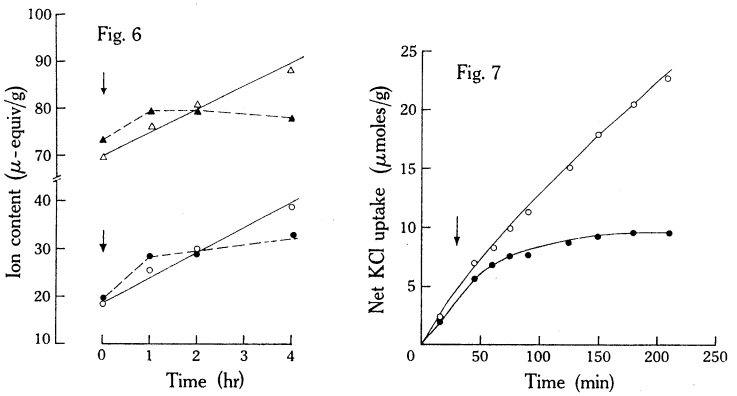

Fig. 6.—Effect of oligomycin on net K^+ and Cl^- uptake by carrot xylem disks aged for 7 days. Disks were incubated for 30 min at 25°C in 40 mM KCl to permit free-space equilibration with the solution before the addition of oligomycin or spectroscopic ethanol (arrows). K^+ (▲,△) and Cl^- (●,○) content of disks was determined for disks incubated at 25°C in 40 mM KCl+0·2% ethanol (open symbols) or 40 mM KCl+0·2% ethanol+12 μg/ml oligomycin (closed symbols).

Fig. 7.—Effect of oligomycin on KCl uptake by beet disks aged for 7 days. The disks were incubated for 30 min at 25°C in 40 mM KCl to permit free-space equilibration with the solution before the start of the experiment. Time of oligomycin or spectroscopic ethanol addition is indicated by the arrow. Net KCl uptake was determined at 25°C by conductivity for disks incubated in 40 mM KCl+0·3% ethanol (○) or 40 mM KCl+0·3% ethanol+6·7 μg/ml oligomycin (●).

In aged carrot xylem disks at 30°C [Figs. 3(*b*) and 4(*c*)], salt respiration is severely inhibited by ethionine, and this decline in salt respiration is associated with a large decline in the ATP level of the aged disks [Figs. 4(*a*), 4(*b*)]. This suggests that salt respiration is in part an ADP-dependent process, rather than being entirely a reflection of increased electron flow coupled directly to anion or cation uptake into mitochondria. Salt respiration can occur in the absence of salt accumulation (Robertson and Thorn 1945), but the experiments shown in Figures 3 and 4 indicate that Rb^+ and Cl^- uptake can continue essentially uninhibited despite severe inhibition of salt respiration. If all the salt respiration was associated with electron flow that was coupled to ion uptake, this partial inhibition of salt respiration by ethionine

would be difficult to explain. However, recent work on the stoichiometries of K^+ movement and ADP–ATP interconversion in isolated animal mitochondria (Cockrell, Harris, and Pressman 1966, 1967) indicates that uptake of 7–10 K^+ and formation of one ATP molecule can be thermodynamically equivalent. If similar conditions exist in intact plant tissue, and if a mechanism exists for coupling of electron transport to salt accumulation, it can be predicted that the oxidative phosphorylation ratio (P/O = 3) for substrates such as pyruvate is equivalent to a ratio of 0·017–0·024 molecules of oxygen reduced per cation accumulated. The residual salt respiration between 50 and 120 min after exposure of carrot slices to ethionine in 40 mM RbCl [Fig. 4(*c*)] was 0·04–0·05 molecules of oxygen reduced per Rb^+ ion accumulated, a value that is compatible with the results for isolated mitochondria but only about 5% of the value commonly found in the absence of ethionine (for a discussion of this ratio see Robertson 1960; Briggs, Hope, and Robertson 1961). The ethionine-sensitive component of the salt respiration—95% from 50–120 min [Fig. 4(*c*)] and eventually 100% when salt uptake had stopped after 120 min [Fig. 3(*b*)]—may be ADP-linked respiration arising from increased activity of salt-stimulated adenosine triphosphatases (Atkinson and Polya 1967); it may also result in part from activation by salt of enzymes involved in oxidative pathways (Evans and Sorger 1966). The results in Section III(*b*) show that both anion and cation uptake can continue linearly at or near control rates despite a concomitant elimination of 50% or more of the ATP in the tissue. This does not necessarily imply an ATP-independence for the uptake process, since an ATP-dependent transport process with a high affinity for ATP would not be inhibited if the residual ATP level, as a result of L-ethionine treatment, was still sufficient to give saturation. The results in Figures 5(*b*) and 5(*d*) indicate that this is an unlikely alternative explanation. Inhibition of terminal oxidation by anaerobic conditions severely inhibited Cl^- uptake into aged carrot xylem disks over 30 min, despite the fact that the tissue ATP level fell by only 30% over the same period. This further supports the observation, based on conductivity measurements, that inhibition of salt uptake precedes ATP depletion in anaerobic conditions (Atkinson *et al.* 1966). Thus inhibition of electron transport prevents salt accumulation, whereas salt accumulation is unaffected by depression of ATP level.

Re-examination of the effect of oligomycin on salt accumulation by aged carrot xylem disks [Section III(*d*); Fig. 6] showed that oligomycin initially failed to inhibit net K^+ and Cl^- uptake for 60 min after application, confirming the observations by Atkinson *et al.* (1966) who found a 30- to 60-min lag before oligomycin inhibited net KCl uptake as measured by conductivity. A shorter lag (15 min) occurs for inhibition of KCl uptake into aged beet disks (Fig. 7). While failure of oligomycin to inhibit ion transport provides strong evidence for direct coupling of ion transport to electron transport, without ATP-mediation in the process, the ultimate inhibition of salt uptake by oligomycin has yet to be interpreted within the framework of a redox pump model (Robertson 1960; Mitchell 1966). ADP has been shown to inhibit swelling and ion transport in mitochondria of rat liver, and it has been suggested that ADP can act as a positive modifier of substrate-induced resistance to mitochondrial swelling (Connelly and Hallstrom 1967). Re-examination of the data of Hodges

and Hanson (1965) on the effect of ADP and oligomycin on ATP-independent Ca^{2+} accumulation by maize mitochondria shows that ADP can inhibit Ca^{2+} accumulation in two ways. ADP+Pi can compete with ion transport for the free energy available from electron transport, and inhibition by this mechanism is oligomycin-sensitive; however, a large proportion of ADP-induced inhibition is not oligomycin-sensitive, and seems to represent an inhibition of transport by ADP *per se*.

Catalytic activities and conformations of many enzymes are regulated by ATP : ADP : AMP ratios (Krebs 1964; Atkinson 1966) and it would be remarkable if artificial changes in these ratios, induced by ethionine or oligomycin, did not have adverse effects, either indirectly or directly, on the conformation of structural proteins, resulting in the eventual leakage of solutes which is characteristic of storage organ disks kept with low ATP levels for long periods. It is suggested from these results that mechanisms of salt accumulation discussed here are independent of ATP but that maintenance of the integrity of structures involved in retention of solutes may be dependent on maintenance of satisfactory ATP : ADP : AMP ratios.

So many of the features of salt accumulation into storage organ disks resemble the characteristics of salt accumulation systems in mitochondria (Hanson 1965; Goh and Wiskich 1967) that there is much interest in the possibility that mitochondria, perhaps in association with other membranous structures that could link redox systems to ion transport, might play a part in salt accumulation. However, two unsolved problems arise in models involving mitochondrial participation in salt accumulation. Firstly, even if active uptake of ions into mitochondria takes place it remains to be explained how this can lead to net salt accumulation in vacuoles. Electron micrographs of plant salt glands (Atkinson *et al.* 1967; Thomson and Liu 1967) show a close association of mitochondria with cytoplasmic membranes, and simple extensions of a number of current chemiosmotic theories (Mitchell 1966, 1967; Robertson 1967, 1968; Slater 1967) may be proposed to account for ion movement in through one side of a mitochondrion and out through the other side when the mitochondrion is associated with a membrane. The possibility that membranes such as the tonoplast contain electron carriers involved in active transport systems does not seem to have been examined adequately. The second problem is the well-known requirement for activation of the cation uptake systems of isolated mitochondria, e.g. for K^+ uptake by valinomycin and for Na^+ and K^+ uptake by gramicidin (for a discussion see Mitchell 1966). It will be interesting to find if there are corresponding endogenous activators involved in the coupling of electron transport to ion transport in plant tissue.

V. Acknowledgments

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