J. Membrane Biol. 74, 51–58 (1983)

The Journal of
Membrane Biology

Chloride Accumulation in Plant Cells as a Homeostatic System: Energy Supply as a Dependent Variable

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Summary. During the accumulation of KCl by excised tissue of the carrot storage root, Cl^- influx falls by 90% or more. The possibility that this reduction is caused by depletion of respiratory substrate and consequent limitation of Cl^- influx by energy supply is disproved. If energy were limiting, then after accumulating KCl (i) the rate of O_2 uptake would be less; (ii) the ATP content might be less; (iii) external glucose and/or sucrose would stimulate O_2 uptake and Cl^- influx; and (iv) respiratory uncouplers would stimulate O_2 uptake less. In each case the opposite was found. The ratio of O_2 uptake in KCl-loaded tissue to that in nonloaded tissue was 1.25 ± 0.21 (7); the ATP concentration was the same after KCl loading; glucose did not stimulate O_2 uptake (except indirectly in the same way as a nonmetabilized glucose analogue) nor did glucose or sucrose stimulate Cl^- influx; and the maximal O_2 uptake in the presence of the uncoupler ClCCP was the same after KCl loading.

The decrease in Cl^- influx must therefore be due to a negative feedback signal from internal Cl^-, as previously suggested. It is pointed out that normal energy supply must be regarded not as an independent, controlling variable; but rather as a dependent variable of the KCl accumulation system.

Conditions where energy supply is or becomes limiting are discussed. The distinction is drawn between pumps operating near equilibrium when energy supply may normally be a controlling factor (e.g., many H^+-ATPases); and other pumps (possibly most of those for the uptake of nutrients and osmotica) which operate far from equilibrium and for which energy supply is a dependent variable.

Key Words chloride flux/accumulation · plant root cells · homeostasis · energy supply · sugar levels · respiration · dependent/independent variables

Introduction

This paper examines the relationship of energy supply to the active transport of ions in higher plant cells. The main conclusion is that energy supply does not limit transport, particularly in pumps far from equilibrium. A picture of a generalized transport system in plants is presented, which includes both signals to transport and its energy supply.

The system investigated experimentally is KCl accumulation by excised tissue of the carrot storage root. During the accumulation of KCl to a final steady level of around 160 µmol (g fresh wt)$^{-1}$ (independent of external KCl concentration – *manuscript in preparation*), Cl^- influx falls by 90% or more. The fall is correlated specifically with the ($Cl^- + NO_3^-$) concentration in the vacuole (Cram, 1973), but the possibility remains that this correlation is not due to a negative feedback signal from the internal ($Cl^- + NO_3^-$) concentration to Cl^- influx, but rather is an indirect relationship.

The alternative proposal (which it is the purpose of this paper to disprove) is that the decrease in Cl^- influx during accumulation is due to limitation by energy supply, due in turn to depletion of respirable sugars (*see*, e.g., discussions in Lüttge & Pitman, 1976; Pitman & Cram, 1977; Cram, 1980*a*). The same hypothesis can be invoked to explain reduced uptake of K^+, $H_2PO_4^-$, and NO_3^- by roots under conditions in which their supply of carbohydrate from the shoot is reduced.

The "energy limitation" hypothesis suffers from the disadvantage of being inconsistent with our knowledge of respiratory control in mitochondria. Respiration is not an overall limitation on the energy-dependent activites of a cell; rather it is a self-regulated activity that automatically matches supply of ATP to demand for its use (Wiskitch, 1980). The cell could only operate with its multitude of energy-dependent, self-regulating and interacting biochemical pathways if their common energy supply were maintained more or less constant and unlimited, though some major, overall regulation of cellular activities, in the direction of synthesis, for example, may have the potential energy supply as an important coordinator (Atkinson, 1972; *see* Discussion).

Nevertheless, it is clear that energy supply may come to limit transport when it (energy supply) naturally, or more frequently artificially, falls towards zero. This has been amply demonstrated in the work designed to characterize energy supply routes by selective inhibition of components of the cell's energy supply system (e.g., MacRobbie, 1970; Raven, 1976). But it does not follow that normal variations in transport rates (of the sort occurring during Cl^- accumulation by plant cells) are also caused by variation in their energy supply.

The first, experimental, part of this paper demonstrates that the change in Cl^- influx during KCl accumulation in carrot cells is *not* controlled by energy supply.

The second part of the paper discusses the general question of what controls normal variations in ion fluxes in plant cells, and, in particular, how energy supply can be included in this picture. The solution offered relies on the distinction between dependent and independent variables. Energy input to the Cl^- transport process is pictured as being a variable that depends on the rate of Cl^- transport, rather than an independent variable that determines it.

Tests of the Hypothesis that Energy Supply Limits Cl^- Transport in KCl-Loaded Carrot Tissue

If a reduced rate of energy supply limits the Cl^- influx in KCl loaded tissue, the following would be predicted:

1) The rate of oxygen uptake would be less in the KCl-loaded tissue.

If the stoichiometry between O_2 uptake and Cl^- influx were tight, then the component of O_2 uptake linked to Cl^- influx would fall by 90% during KCl accumulation. This component will be taken as being the 25% stimulation of respiration in water-washed tissue when it is placed in KCl solution (e.g., Robertson & Wilkins, 1948, and similar values observed in the present experiments). The predicted decrease in O_2 uptake rate would then be $0.9 \times 0.2 = 0.18$ (18%) of the rate in water-washed tissue in 1 mM KCl.

2) The ATP level would be less in KCl-loaded tissue, if Cl^- influx depends directly or indirectly on energy supplied via ATP.

If the pump rate were proportional to the ATP concentration, then in KCl-loaded tissue the ATP concentration would be 10% of its value in non-loaded tissue. In *Neurospora* the electrogenic pump has a rectangular hyperbolic relationship to ATP concentration *in vivo*, with a $K_{\frac{1}{2}}$ of about 2 mM, which is comparable to other ATP-dependent pumps (Slayman, Long & Lu, 1973). If the same were true of carrot then the ATP level would have to fall to less than 10% of the control level to cause a 90% fall in Cl^- influx in the KCl-loaded tissue.

3) Supplying external glucose and/or sucrose would stimulate both the rate of oxygen uptake and Cl^- influx in KCl-loaded tissue if the supply of respiratory substrate were limiting.

For glucose in the cytoplasm to limit respiration, it must be present at concentrations comparable to the $K_{\frac{1}{2}}$ for hexokinase (around 0.1 mM – Turner & Turner, 1975; Turner & Copeland, 1981). In the absence of external glucose this must be supplied by hydrolysis of sucrose or influx to the cytoplasm from the vacuole. From the cytoplasmic volume (about 2% of the tissue volume, or 0.02 ml g fresh wt^{-1}) the cytoplasmic glucose content must be around 0.1 μmol $ml^{-1} \times 0.02$ ml g fresh wt^{-1} or 0.002 μmol g fresh wt^{-1}. From the rate of O_2 uptake the rate of utilization of glucose in respiration can be calculated to be 3/6 or 0.5 μmol g fresh $wt^{-1}hr^{-1}$. For glucose turnover in the cytoplasm the rate constant must therefore be $0.5/0.002 = 250\ hr^{-1}$, giving a half time of 0.2 min. If glucose supply limits respiration then it follows from the calculations above that an additional glucose influx to the cytoplasm of 3.5 μmol g fresh $wt^{-1}hr^{-1}$ from 10 mM externally supplied glucose (Grant & Beevers, 1964) would increase the rate of O_2 uptake by 100% (hexokinase activity would be saturated) within minutes and would increase Cl^- influx similarly if it is limited by energy supply from respiration.

Externally supplied sucrose, which is hydrolyzed to UDPG and fructose or glucose and fructose *in vivo*, would have an effect similar to externally supplied glucose.

4) Oxygen uptake in KCl-loaded tissue would be stimulated less by respiratory uncouplers, and the maximally uncoupled O_2 uptake rate would be significantly less in the KCl-loaded tissue.

Materials and Methods

Phloem or xylem tissue of the storage root of carrot (*Daucus carota* L) and tissue of red beet (*Beta vulgaris* L) was cut into rectangles approximately 5×5×1 mm and washed in aerated distilled water for several days. After 4 or 5 days part of a batch of tissue was allowed to accumulate ions from 10 mM KCl+0.1 mM $CaSO_4$ for a further 4 to 5 days. At this stage the cellular KCl level would be about 120 μmol g^{-1}, which is 80% of the final steady 160 μmol g^{-1} that would be accumulated after 10 days (Cram, 1980*b*).

Chloride influx was estimated using ^{36}Cl. The tissue was placed in ^{36}Cl-labeled solution for 30–60 min and then extracellular $^{36}Cl^-$ removed by washing in nonlabeled solution. Details are given with the relevant tables.

Oxygen uptake rates were measured in a Warburg respirometer since fairly long term measurements were required. Initial oxygen uptake rates were measured in a standard solution of 1 mM KCl+0.1 mM $CaSO_4$, and then the solution was changed and the O_2 uptake followed until a new steady rate was reached. A correction was made for drift with time (a fall of 20% or less over 8 hr in controls).

ATP was assayed by the method used by Pitman, Wildes, Schaefer and Wellfare (1977).

Results are expressed as mean ± SEM (no. of replicates), unless the SD is explicitly referred to.

Fluxes and contents are all expressed as per gram fresh weight.

Results

1. *The Rate of Respiration in KCl-Loaded Carrot Tissue*

The rates of O_2 uptake in KCl-loaded and in nonloaded tissue were measured several times in these experiments. The average uptake rates in 1 mM KCl+0.1 mM $CaSO_4$ were

$$qO_2 \text{ in nonloaded tissue} = 2.4 \pm 0.2\ \mu\text{mol g}^{-1}\text{hr}^{-1}\ (7, \text{SD})$$

$$qO_2 \text{ in KCl-loaded tissue} = 3.0 \pm 0.5\ \mu\text{mol g}^{-1}\text{hr}^{-1}\ (7, \text{SD}).$$

Similar results have previously been obtained for red beet by Sutcliffe (1952).

The ratio of the rate in KCl-loaded to that in nonloaded tissue from the same carrot roots was

$$\frac{qO_2 \text{ in KCl-loaded tissue}}{qO_2 \text{ in nonloaded tissue}} = 1.25 \pm 0.21\ (7, \text{SD}).$$

The O_2 uptake rate in KCl-loaded tissue is therefore significantly greater than in nonloaded tissue, and not 20% less as predicted by the respiratory limitation hypothesis.

Extending these observations, Cl^- influx and O_2 uptake rates were also measured in carrot and beet tissue that had been allowed to accumulate salt from KNO_3, NaCl, K_2SO_4+$KHCO_3$, and $CaCl_2$. Figure 1 shows that in both carrot and beet there was no correlation between Cl^- influx and O_2 uptake rate after loading with these various salts.

2. *ATP Contents of KCl-Loaded and Nonloaded Carrot Tissues*

Table 1 shows that the ATP content is the same in KCl-loaded and in nonloaded tissues. Although the variability is quite high, and the expected difference in Cl^- influx is in this case about threefold rather than the more usual tenfold, the probability that the smaller Cl^- influx after KCl loading is accounted for by a fall in ATP concentration in this batch of tissue is less than 2%, and the possibility will therefore be discounted.

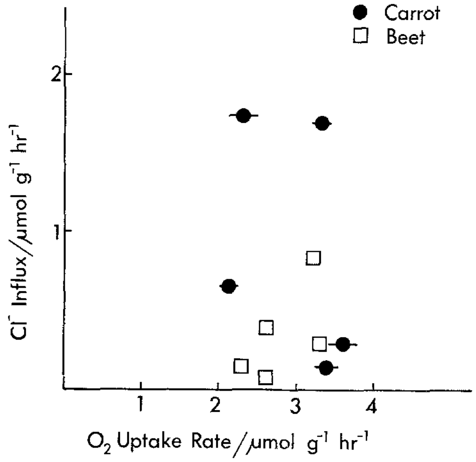

Fig. 1. The independence of Cl^- influx from respiration rate in carrot and red beet root storage tissue. Tissue was aged in aerated water after cutting and then allowed to accumulate ions from a range of salt solutions for several days, as described in the text. Cl^- influx is reduced to varying extents after the different loading treatments. Oxygen uptake rates also vary, but Cl^- influx is not correlated with O_2 uptake rate. Bars mark ±1 SEM. Where it is not shown, this lies within the symbol

Table 1. ATP contents of KCl-loaded and nonloaded carrot tissue[a]

Pretreatment	ATP content (μmol g^{-1})	Cl^- influx (μmol g^{-1} hr^{-1})	Cl^- content (μmol g^{-1})
H_2O	16.3±1.3 (3)	0.34±0.00	22±1
KCl load	16.6±3.3 (3)	0.13±0.00	124±2

[a] Tissue was washed for 10 days in aerated H_2O or for 5 days in H_2O, 5 days in 10 mM KCl+1 mM $CaSO_4$ and 1 day in H_2O. ATP content, Cl^- influx and Cl^- content were then measured in samples of the two batches.

3. *(a) Effects of Changes in Vacuolar Sugar Concentrations during KCl Accumulation*

During the accumulation of KCl there is a progressive fall in the concentration of reducing sugars in carrot (Cram, 1980*b*). As shown above, there is no corresponding decrease in the O_2 uptake rate. Figure 2 shows further that in tissue that had accumulated ions from a number of other salt solutions (the experiment referred to in section 1 above)

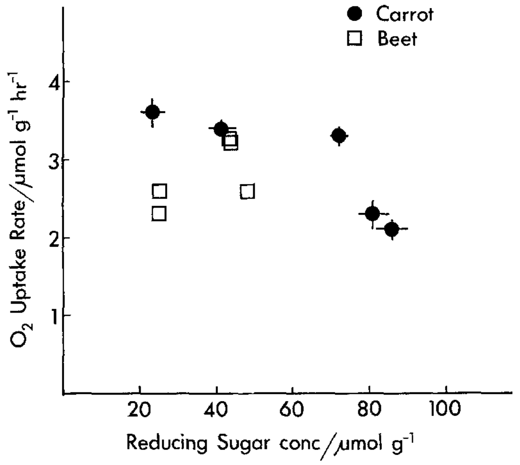

Fig. 2. Independence of respiration rate and reducing sugar concentration in carrot and red beet tissue. In the same experiment as that shown in Fig. 1 the cellular concentration of reducing sugars was also measured. Oxygen uptake varies independently of the reducing sugar concentration (which must be the concentration in the vacuole since this occupies over 90% of the volume of the cell). Vacuolar sugars are not a limiting factor in the rate of respiration in these tissues

Table 2. The effect of external glucose and 2-deoxy-D-glucose on O_2 uptake rates by carrot tissue[a]

Pretreatment	Oxygen uptake rates (μmol g^{-1} hr^{-1})		
	Control	+10 mM glucose	+10 mM 2-deoxy-D-glucose
A H_2O	2.5±0.1 (10)	3.3±0.1 (5)	3.4±0.1 (5)
KCl load	2.6±0.1 (10)	3.4±0.2 (5)	2.9±0.1 (5)
B H_2O	1.6±0.1 (4)	2.8±0.1 (8)	2.8±0.2 (8)

[a] Carrot tissue was washed for 8 days in H_2O or 4 days in H_2O plus 4 days in 10 mM KCl+1 mM $CaSO_4$. In A, oxygen uptake rates were measured in 1 mM KCl+0.1 mM $CaSO_4$ and then glucose or 2-deoxy-D-glucose was introduced from the side arm and oxygen uptake followed until a new steady rate was reached, which was in less than 30 min. The percentage stimulation by glucose is not significantly different from the stimulation caused by 2-deoxy-D-glucose. In B, the rates were measured simultaneously in separate samples.

there was no correlation between vacuolar reducing sugar levels and O_2 uptake rates.

(b) Effects of External Glucose and Sucrose on O_2 Uptake and Cl^- Influx

It is in fact not the vacuolar but the cytoplasmic sugar concentration which directly supplies respiration. It was calculated in the introduction that supplying external glucose at 10 mM would relieve any respiratory substrate limitation on respiration if it occurred.

Table 3. The effect of external glucose on Cl^- influx in carrot tissue[a]

Pretreatment	Cl^- influx (μmol g^{-1} hr^{-1})	
	−glucose	+10 mM glucose
H_2O	0.67±0.01	0.65±0.02
KCl load	0.30±0.00	0.30±0.00

[a] Tissue was washed for 11 days in H_2O or for 7 days in H_2O plus 4 days in 10 mM KCl+1 mM $CaSO_4$ and then 5 hr in H_2O. Cl^- influx from 2 mM KCl+0.1 mM $CaSO_4$±10 mM glucose was then measured over 60 min followed by a 30-min wash in inactive solution. Results are quoted to two decimal places

Table 2 shows that supplying 10 mM glucose in the external solution stimulates O_2 uptake in both KCl-loaded and in nonloaded tissues. The percentage stimulation is about the same in the two. However, 2-deoxy-glucose (which is also taken up but is not metabolized) stimulates respiration to about the same extent as does glucose in both KCl-loaded tissue (Table 2*A*) and in nonloaded tissue (Table 2*A* and *B*). The stimulation by sugars is comparable to that caused by KCl itself in nonloaded tissue. Glucose therefore probably stimulates O_2 uptake not by increasing the respiratory substrate concentration but rather by the same mechanism as does KCl (by drawing on energy for transport – to anticipate the final conclusion of this paper). A similar stimulation of O_2 uptake by nonmetabolized glucose analogues in *Chlorella* has been observed by Decker and Tanner (1972).

Neither 10 mM external glucose (Table 3) nor 10 mM external sucrose (results not shown) stimulate Cl^- influx in either KCl-loaded or nonloaded carrot tissue, confirming that the reduced influx in KCl-loaded tissue is not due to substrate limitation of respiratory energy supply.

4. Effects of the Respiratory Uncoupler ClCCP

ClCCP stimulates the rate of O_2 uptake by 60 to 80% in KCl-loaded tissue and in nonloaded tissue. The new rate is reached within 30 min. As shown in Fig. 3, the maximum O_2 uptake rate is reached at about 10^{-6} M ClCCP. Figure 3 also shows that O_2 uptake rates in KCl-loaded and in nonloaded tissues are the same. Six independent sets of measurements of the O_2 uptake rate in loaded and nonloaded tissues in 10^{-6} to 10^{-5} M ClCCP gave the ratio

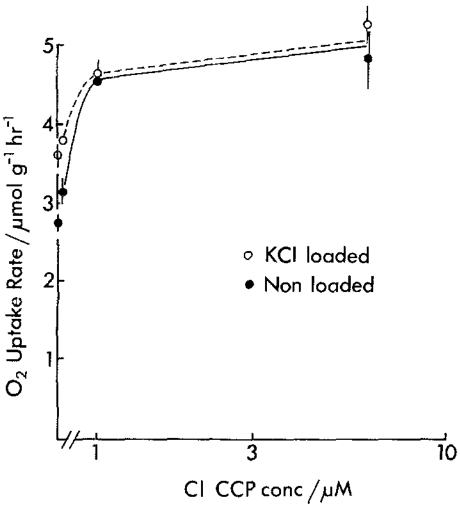

Fig. 3. Stimulation of oxygen uptake by the respiratory uncoupler in KCl-loaded and in nonloaded carrot tissue. In both tissues the rate of oxygen uptake is stimulated by ClCCP, and the maximally stimulated rate is the same in the two (*see also* Results in the text). This indicates that there is no limitation by respiratory substrate of the rate of respiration in nonloaded or in KCl-loaded tissue. Experimental details in the text. Bars show ±1 SEM

$$\frac{\text{Maximum uncoupled } qO_2 \text{ in KCl-loaded tissue}}{\text{Maximum uncoupled } qO_2 \text{ in nonloaded tissue}} = 1.04 \pm 0.11 \ (6, \text{SD}).$$

The substantial stimulation by ClCCP, and the fact that the maximally uncoupled rates are the same in KCl-loaded and nonloaded tissues, shows that there is no limitation of respiration by substrate supply in either type of tissue.

Discussion

The results presented in this paper clearly disprove the hypothesis that a limitation of energy supply accounts for the reduction in Cl^- influx during KCl accumulation in carrot tissue. None of the four predictions of the hypothesis were fulfilled. After KCl loading the oxygen uptake rate is faster (not slower); the ATP concentration is the same (not less); external glucose does *not* stimulate Cl^- influx, and its slight effect on respiration is probably indirect; and with the uncoupler ClCCP the maximally stimulated O_2 uptake rate is the same as in nonloaded tissue (not less). In KCl-loaded tissue, therefore, respiratory substrate cannot be limiting respiration, and respiration cannot be limiting Cl^- influx.

The reduction in Cl^- influx during KCl loading is therefore most probably the result of a negative feedback signal related to the approach to some reference value of internal Cl^- concentration, as previously suggested (Cram 1973, 1976, 1980*b*, and by implication, Sutcliffe, 1952).

It is, however, as well not to overlook the fact that there are conditions when energy supply does limit transport. These will be discussed after presenting an alternative picture of how energy supply is related to the other components of the Cl^- transport system.

Energy Supply as a Dependent Variable

We must first distinguish between dependent and independent variables in the Cl^- transport system. External Cl^- concentration can be changed to a new value quite independently of the rest of the system, as is done in any kinetic experiment or fertilizer treatment. External Cl^- concentration is thus an *independent* variable, and changes in it simply perturb the Cl^- accumulation system (whose negative feedback properties ensure that the same final level of accumulated Cl^- is reached eventually) (Cram, 1976, 1980*b*[1]).

An example of a dependent variable could be seen in an ATP-dependent ion transport process functioning in a cell in which oxidative phosphorylation is limited by ADP availability. (This should not be taken to imply that Cl^- influx is directly ATP-dependent: its mechanism is not yet established). When the ion flux increases, for instance following an increase in the external ion concentration, ATP will be hydrolyzed at a greater rate, the ADP concentration will rise, and the rate of ATP production by the mitochondria will increase until it matches the rate of utilization by the pump.

In this hypothetical system, therefore, ATP production does not control ion flux, but rather it is dependent on the ion flux itself and can be called a *dependent* variable.

The response of oxidative phosphorylation to ADP gives it the superficial appearance of a negative feedback system, as increased output (ATP concentration) decreases the rate of ATP production. However, there is no independent regulating signal, and it cannot be put in the same class as a *bona fide* feedback system. For a discussion of this aspect *see*, e.g., Toates, 1975.

This is presented in block diagram form in Fig. 4. The simple feedback loop suggested previously (Cram, 1976) consists of a signal comprising the difference between the actual Cl^- concen-

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[1] Also W.J. Cram (*manuscript in preparation*).

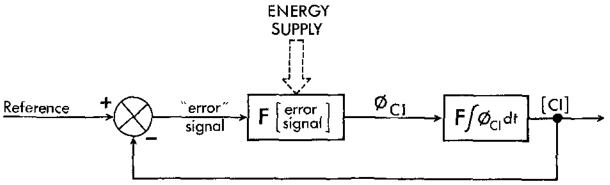

Fig. 4. Block diagram of the Cl^- uptake and accumulation system in carrot storage root cells to show the relationship between feedback signals and energy input to Cl^- influx. Internal Cl^- concentration ([Cl]) is determined by Cl^- influx integrated over a period of time, together with Cl^- passive leaks and the volume of the cell. These are represented by the right-hand box in which *F* stands for 'function of.' Cl^- influx itself receives a feedback signal from internal Cl^- concentration, whose size is proportional to the difference between an inbuilt reference value and the actual value of Cl^- concentration. Chloride influx is a function of this 'error' signal (and of other inputs not shown here), but is *not* a function of the energy supply, which for this reason is drawn as a ghost arrow

tration in the cell and some inbuilt reference value. This flows back to dictate the instantaneous value of Cl^- influx. Continuing Cl^- influx causes the internal Cl^- concentration to rise until the reference value is approached.

Energy flows to the transport process independently of the feedback signal. Since it does not carry any independent information to dictate the value of Cl^- influx it does not influence the dynamic characteristics of the system, and could appropriately be omitted from the diagram. It is drawn with dashed lines to distinguish it from other flows. For further discussion of energy flows in feedback systems *see* Milsum (1966) and Jones (1973).

Energy and Information in Other Plant Root Systems

The only accumulation system considered so far is that for Cl^- in excised carrot tissue. The same relationship of uptake to energy supply probably applies also to Cl^- accumulation in excised barley and maize roots (Cram, 1973). It should be re-emphasized that during KCl accumulation by these tissues the progressive fall in sugar concentrations accompanies, but, as this paper shows, does not cause the progressive fall in Cl^- influx.

However, there are observations on roots in intact plants where changes in sugar concentrations do seem to cause changes in ion fluxes, and the energy limitation hypothesis has been invoked in explanation. These are the reduced uptake of K^+, $H_2PO_4^-$, and NO_3^- by roots and their reduced transport into the xylem after the shoots have been darkened to reduce photosynthesis, the stem ringed or cooled to eliminate transport of carbohydrate in the phloem to the root, or the root excised (e.g., Bange, 1965; Pitman, 1972; Bowling & Dunlop, 1978; Jackson, Volk & Israel, 1981; Jeschke, 1982). The time course of these effects and their reversal by adding extracellular sugars is consistent with the hypothesis of energy limitation, but it seems unlikely that these intact roots differ from carrot tissue in so fundamental a manner, and therefore another hypothesis is worth considering.

It would appear essential for the plant root to respond to the shoot, in particular to the actual or potential growth rate as evidenced by the rate of photosynthesis or carbohydrate supply to the root. What the above experiments on roots may be revealing, therefore, is a signal from the shoot to the root, directly or indirectly related to the transport of carbohydrate or its accumulation in the root. An informational role for sugar levels is not a new concept: sucrose levels have, for instance, been implicated in determining the course of differentiation in callus and stem tissues (e.g., Northcote, 1971). More generally, the point that potential energy supply may constitute a signal as well as a driving force has been made previously in several guises (Atkinson, 1972; Cram, 1976; Raven, 1976).

Energy Supply as an Independent Variable

We must still consider whether pumps can be controlled (sometimes in the sense of restricted rather than of regulated) by energy supply.

Clearly in the limit (dark, N_2 atmosphere) when photosynthesis and respiration are both operating at zero rate, though glycolysis may continue, energy-dependent fluxes will be strongly inhibited. The active flux will fall towards a stage when the free energy gradient of the actively transported substance balances the free energy available from the driving reaction. In other words, the pump will be near equilibrium.

In this region there may be a linear relationship between ion flux and the rate of the metabolic reaction to which it is coupled. The near equilibrium state has been considered theoretically within the formalism of irreversible thermodynamics (e.g., Katchalsky & Curran, 1965; Walker, 1976). With reciprocal coupling, a change in the potential energy supply may now cause a proportional change in active flux (and *vice versa*), so that energy supply may become an independent variable.

When the same pumps are operating normally away from equilibrium, a linear dependency of flux on metabolic driving force can no longer be assumed. The characteristics of the relevant coupling coefficient (L_{ir}, to follow the convention used by Katchalsky & Curran, 1965) can be inferred from observation or mechanism. The frequently observed saturation by ATP of transport ATPases (Slayman et al. 1973) suggests a zero order reaction between energy supply and a pump operating far from equilibrium (*see also* the discussion in Hansen, Gradman, Sanders and Slayman (1981)). The coupling coefficient L_{ir} would then be zero, which is an alternative way of stating the main contention of this paper.

Salt respiration (the stimulation of respiration by adding external salt (Robertson & Wilkins, 1948; Robertson, 1968)) implies that the reverse coupling coefficient (L_{ri}) connecting energy supply and flux, is simultaneously nonzero. (At the same time it is puzzling to find that the feedback inhibition of salt influx does not appear to have reverse effect.) Since the system is not near equilibrium this inequality of reciprocal coupling coefficients does not violate Onsager's theorem (e.g., De Groot, 1963).

The difference between dependent and independent variables also appears in other analytical approaches such as that of reaction kinetics, which may be more applicable than irreversible thermodynamics to multicomponent processes such as transport across a membrane (e.g., Sanders, 1983).

The exact stage at which inhibiting the potential energy supply begins to limit the transport process coupled to it, and the stage at which proportionality between flux and energy supply are reached, are known in only a few cases (e.g., Slayman et al., 1973; Steinmetz & Andersen, 1982). This is an uncertainty in the interpretation of experiments designed to show up linkages between fluxes and energy supply by progressively inhibiting the latter. (*See also* the discussion by Raven (1976) and other papers referred to therein and Sanders, 1983).

In terms of Fig. 4, when energy supply becomes limiting it becomes an independent variable which can perturb the system. Feedback and other signals will act so that the system will continue to operate as a homeostat. In this condition a change in the potential energy supply will alter the instantaneous influx, but not the final level accumulated. This is the simplest interpretation of the observations that ClCCP reduces Na^+ and K^+ influx to barley root cells, but affects the final level accumulated much less (Jeschke, 1974).

Pumps Close to or Far from Equilibrium

For completion we must also point out that in contrast to the Cl^- pump in carrot there are some pumps whose function and efficiency demands that they normally operate near equilibrium. The main ones are the H^+-pumping ATPases found in several membranes in diverse cells (e.g., Steinmetz & Andersen, 1982). The rates of these pumps will normally depend on the potential rate of energy supply. It may be possible to distinguish between, on the one hand, possibly the majority of pumps operating far from equilibrium, which provide specific substances to compartments of the cell and are normally "oversupplied" with energy (e.g., Cl^-, NO_3^-, etc., in plants), and, on the other hand, the relatively fewer pumps operating close to equilibrium, whose rates depend on the potential energy supply (e.g., H^+ in plants and animals). The former systems have energy as a dependent variable; the latter systems have energy as an independent variable.

I am grateful to Robert Reid for the ATP assays and to Dale Russ for technical assistance, to Alan Walker and Dale Sanders for helpful discussion on theoretical aspects of the work, and to the Australian Research Grants Committee and the University of Sydney for financial support.

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Received 9 August 1982; revised 12 November 1982