Interactions between growth, Cl⁻ and Na⁺ uptake, and water relations of plants in saline environments. I. Slightly vacuolated cells

H. GREENWAY,* RANA MUNNS† & JOE WOLFE†‡ *Department of Agronomy, Institute of Agriculture, University of Western Australia, Nedlands, WA 6009, Australia; and †CSIRO, Division of Plant Industry, PO Box 1600, Canberra City, ACT 2601, Australia

Abstract. Slightly vacuolated cells, i.e. microalgae and meristematic cells of vascular plants, maintain low Cl⁻ and Na⁺ concentrations even when exposed to a highly saline environment. The factors regulating the internal ion concentration are the relative rate of volume expansion, the membrane permeability to ions, the electrical potential, and the active ion fluxes.

For ion species which are not actively transported, a formula is developed which relates the internal concentration to the rate of expansion of cell volume, the permeability of membranes to that ion, and the electrical potential. For example, when the external concentration of Cl⁻ is high, and Cl⁻ influx is probably mainly passive, the formula predicts that rapid growth keeps the internal Cl⁻ concentration lower than that in a non-growing cell with the same electrical potential; this effect is substantial if the plasmalemma has a low permeability to Cl⁻.

For ion species which are actively transported, the rate of pumping must be considered. For instance Na⁺ concentrations are kept low mainly by an efficient Na⁺ extrusion pump which works against the electric field across the membrane. The requirement for Na⁺ extrusion is related to the external Na⁺ concentration, the rate of expansion of cell volume, the membrane permeability, and the electrical potential. It is possible that microalgae have a more positive electrical potential than many other plant cells; if so, requirements for high rates of active Na⁺ extrusion will be lower. The required rates of Na⁺ extrusion are lower during rapid growth, provided that the permeability of the plasmalemma to Na⁺ is low.

The energy required for the regulation of Cl⁻ and Na⁺ concentrations is low, especially in rapidly expanding cells where Na⁺ extrusion requires only 1–2% of the energy normally produced in respiration. The exclusion of these ions, however, must be accompanied by the synthesis of enough organic compounds to provide adequate osmotic solutes for the increases in volume accompanying growth. This process reduces the substrates available for respiration and synthesis of cell constituents, but the reduction is not prohibitively large—even for cells growing in 750 mol m⁻³ NaCl, the carbohydrate accumulated as osmotic solute is only 10% of that consumed in respiration.

Introduction

This and the subsequent article consider some aspects of the interactions between growth, ion relations, and water relations of plants living in marine and terrestrial saline environments. We define saline habitats as those containing water with an osmotic pressure of more than 0.33 MPa, equivalent to 70 mol m⁻³ monovalent salts (Greenway & Munns, 1980). The ion concentration in ocean waters is approximately (mol m⁻³): $460\,Na^+$, $10\,K^+$, $10\,Ca^{2+}$, $50\,Mg^{2+}$, and $540\,Cl^-$. This gives an osmotic pressure of 2.5 MPa (Levring, Hope & Schmid, 1969).

Opinions differ on the levels of Cl⁻, Na⁺, and K⁺ which seriously inhibit metabolism. However, the available information on *in vitro* enzyme activity definitely indicates that metabolism would be adversely affected once Cl⁻ and (Na⁺ + K⁺) concentrations exceed 300–400 mol m⁻³ (Jennings, 1976; Munns, Greenway & Kirst, 1982). Furthermore, K⁺/Na⁺ ratios of less than 1.0 are probably inhibitory to some metabolic processes, including protein synthesis (Greenway & Munns, 1980; Wyn Jones, Brady & Speirs, 1979). This conclusion is consistent with estimates of internal ion concentrations of most freshwater and marine microalgae. Cellular ion concentrations of such species are likely to be within these limits, even when cells are exposed to high salinity (Table 1). Na⁺ concentrations in salt lake species, such as *Dunaliella parva*, are possibly higher than 300–400 mol m⁻³, but are still substantially lower than Na⁺ in the

Correspondence: Dr H. Greenway, Department of Agronomy, University of Western Australia, Nedlands, WA 6009, Australia.

‡Present address: School of Physics, University of New South Wales, PO Box 1, Kensington 2033, Australia.

Table 1. Internal ion concentrations (mol m^{-3}) in microalgae growing at high salinity. A more extensive table is given in Munns et al. (1982)

External solution		Chlorella salina* (marine)			Platymonas subcordiformis* (marine)			Dunaliella parva† (salt lake)		
NaCl	K$^+$	Cl$^-$	Na$^+$	K$^+$	Cl$^-$	Na$^+$	K$^+$	Cl$^-$	Na$^+$	K$^+$
100	3.5	—	—	—	20	20	210	—	—	—
200	3.5	10–20	10–20	100	—	—	—	—	—	—
500	3.5	10–20	10–20	70	70	150	400	—	—	—
1500	1.0	—	—	—	—	—	—	—	700‡	100‡

*Kirst (1977a, b).

†Gimmler & Schirling (1978).

‡The high Na$^+$/K$^+$ in D. parva (cf. P. subcordiformis) may be related to the high Na$^+$/K$^+$ in the external solution in this experiment rather than to inherent factors.

external solution (Table 1). Because internal concentrations of Cl$^-$ and Na$^+$ of marine and salt lake microalgae are much lower than those in the external solution, organic solutes contribute a large part of the total internal solute concentration (*see reviews by* Kauss, 1978; Munns et al., 1982). The same principle probably applies to the cytoplasm of highly vacuolated cells of terrestrial halophytes (Munns et al., 1982). The principal aim of this paper is to discuss mechanisms by which plant cells keep low Cl$^-$ and Na$^+$ concentrations in their cytoplasm, when the cells are exposed to high external NaCl concentrations.

In slightly vacuolated cells, i.e. microalgae and the meristematic cells of higher plants, the internal ion concentration will be low if the rate of cell expansion is sufficiently high, and the membrane permeability to ions is low. The influx of Cl$^-$ and Na$^+$ at high external concentrations is presumed to be mainly passive. The fact that nearly all plant cells have a negative electrical potential implies that an active Na$^+$ efflux is essential, and that active Cl$^-$ efflux is much less likely. The influence of these fluxes, as well as of the growth rate and the membrane permeability to ions, on the internal ion concentration, is the theme of this paper. It is assumed, for simplicity, that in slightly vacuolated cells, solutes other than Cl$^-$ and Na$^+$ produce a high internal solute content so that sufficient turgor (or volume, in the case of wall-less cells) is generated for growth. In the subsequent paper, the somewhat different case of highly vacuolated cells is treated: in such cells, ions are the major solutes contributing to turgor generation. The relative energy costs of these alternatives, i.e. the use of organic solutes versus ions for turgor-volume maintenance in a saline environment, are analysed in the conclusion of the subsequent paper.

The relation between ion concentrations and growth rates in a system at steady state

The assumption that a system is in a steady state implies that the concentrations and fluxes of all species, including water, are constant. This assumption simplifies considerably an analysis of the relation between growth rates and ion concentrations. The index of growth we shall consider is volumetric expansion, and we shall restrict our analysis to expansion in the steady state. We acknowledge that, in general, growth of walled cells may be accompanied by changes in concentration; nevertheless, the steady state does approximate the condition of cells in the exponential phase of growth. This approach is similar to that used by Jennings (1969) in considering uptake of essential nutrients.

If the internal concentration of some ion, $[X]_i$, is unchanged during expansion, then the ratio of the increase in the total number of ions to the increase in the amount of water must be the same as the ratio of the number of those ions in the cell to the amount of water in the cell already, i.e.

Net rate of ion uptake = $[X]_i \cdot$ [net rate of water uptake].

Now if V is the volume of water in the cell:

$$\text{Total rate of ion uptake} = [X]_i \frac{dV}{dt} \qquad (1)$$

(This is equivalent to Eqn. 7 of Jennings (1969) for the case of the steady-state assumption, i.e. $(d[X]_i/dt) = 0$).

Chloride fluxes in steady state

Unless otherwise stated, we shall assume that at high $[Cl^-]_o$, the Cl$^-$ flux is passive, i.e. it is determined solely by concentration gradients, electric field, and membrane permeability. It should be emphasized that this assumption would apply, at best, to high Cl$_0^-$; active Cl$^-$ *influxes* have been well established for several species of microalgae grown at Cl$^-$ less than 10 mol m^{-3} (reviewed by Raven, 1980). As far as we know, Cl$^-$ *efflux* is always entirely passive (Raven, 1976). With this assumption, and assuming a uniform electric field across the membrane (i.e. negligible dielectric variation or charge in the

membrane), we may employ the passive flux equation from Goldman as given by Briggs, Hope & Robertson (1961) (their Eqn. 30):

$$J = -zFE/RT \cdot P_X \cdot \frac{[a_0 - a_i \exp{(zFE/RT)}]}{1 - \exp{(zFE/RT)}}, \quad (2)$$

where J is the net inward flux (per unit area), z is the electric charge of the ion, F is the charge of one mol of protons, E is the electrical potential of the inside of the membrane less that of the outside, P_X is the permeability of the membrane to the ion X, R is the gas constant, T the thermodynamic temperature and a_i and a_0 are the activities of ion inside and outside the membrane, respectively. We may rewrite Eqn. 1 in terms of J for a cell of surface area A:

$$J = [X]_i \left(\frac{1}{A} \cdot \frac{dV}{dt} \right). \quad (3)$$

Making the further assumption that the activity of ions is equal to their concentration, we may rearrange Eqns 2 and 3 to relate cell expansion rate at steady state to the internal and external concentration of chloride ions:

$$\frac{[X]_i}{[X]_0} = \frac{\exp{(-zFE/RT)}}{1 + \left(\frac{1}{P_X} \cdot \frac{1}{A} \cdot \frac{dV}{dt} \right) \frac{1}{zFE/RT} [1 - \exp{(-zFE/RT)}]}. \quad (4)$$

For spherical cells, $[(1/A)(dV/dt)]$ is appropriate since it is approximately equal to the rate of radial expansion. However, the relative expansion rate (G) is more usually measured, and in the steady state,

$$G = \frac{1}{V} \frac{dV}{dt}.$$

Now

$$\frac{1}{A} \frac{dV}{dt} = \frac{V}{A} \cdot G$$

and for Cl^-, $z = -1$. Thus Eqn. 4 becomes

$$\frac{[Cl^-]_i}{[Cl^-]_0} = \frac{\exp{(FE/RT)}}{1 - \left(\frac{1}{P_{Cl^-}} \cdot \frac{V}{A} \cdot G \right) \frac{1}{FE/RT} \cdot [1 - \exp{(FE/RT)}]} \quad (5)$$

Equation 5 has more than one unknown. P_{Cl^-} and E, for example, are unknown for microalgae grown in high [NaCl]. An indication of P_{Cl^-} can be gained from data obtained for the plasmalemma of freshwater macroalgae: P_{Cl^-} of Charophytes varies between 2×10^{-12} and 4×10^{-11} m s^{-1} (Raven, 1976). Measurements of E of freshwater microalgae with microelectrodes have given an E of -40 mV for *Chlorella pyrenoidosa* grown in 1 mol m^{-3} Cl^- (Barber, 1968) and of -55 mV for *Chlorella fusca* in

0.01 mol m^{-3} Cl^- (Langmüller & Springer-Lederer, 1974). This potential is rather smaller than the potentials (typically -140 mV) measured in the vacuoles of highly vacuolated cells. If this difference is real, rather than artefactual, then it means either that the potential across the plasma membrane is lower in slightly vacuolated cells than in highly vacuolated cells, or that a substantial potential difference exists across the tonoplast of highly vacuolated cells. The difference may be artefactual, however: the insertion of microelectrodes into microalgae may cause short-circuiting resulting in a recorded E which would be much less negative than the E of the cell (Davis, personal communication). A much more negative E of -135 mV was estimated for a freshwater species, *Chlorella vulgaris*, using positively charged lipophilic compounds, labelled with ^{3}H (Komor & Tanner, 1976). This value is closer to the E usually found in freshwater macroalgae (Raven, 1976), but results obtained with these lipophilic cations also remain in some doubt, due to several unverifiable assumptions (Davis, personal communication). In any case, E might depend on experimental conditions and metabolic activity (Pitman & Lüttge, 1982).

A further variable which should be considered is a change in P_{Cl^-} with increasing salinity. Passive movement through the lipid bilayer of the membrane would be very small, P_{Cl^-} of polar lipid bilayers being 1×10^{-12} m s^{-1} (Raven, 1976). Most of the passive Cl^- movement will therefore be via porters or channels (Raven, 1980). If the Cl^- flow is via porters, then rates of uptake may become saturated at high salinity, and the *apparent* P_{Cl^-} will decrease with further increases in $[Cl^-]_0$. On the other hand, if Cl^- flows mainly through ion channels, then a change in membrane structure may cause a *real* change in ion permeability.

Despite our ignorance of the values of the parameters in Eqn. 5, and of the undoubtedly complicated interactions between them, we wish to examine the dependence of $[Cl^-]_i$ on $[(1/V)(dV/dt)]$, with all other parameters fixed. We shall express this as the dilution of internal $[Cl^-]$ due to expansion; that is, the ratio of $[Cl^-]_i$ in a cell with a given growth rate to $[Cl^-]_i$ in a cell with constant volume, but with the same E and $[Cl^-]_0$ as the growing cell. This dilution is shown in Fig. 1 for different values of E. Strictly speaking, the conditions demanded in our comparison are not found in practice; with the exception of a voltage clamp it is difficult to imagine an electrogenic pump which could maintain E when $[Cl^-]_i$ is varied. Figure 1A shows, however, that provided the variation in E is small ($\leqslant 10$ mV, say), the dilution effect of expansion is well approximated by Eqn. 5. Figure 1A shows that growth rates of 30–40×10^{-6} s^{-1} would substantially reduce $[Cl^-]_i$; and such growth rates have been recorded for some species of microalgae (Munns *et al.*, 1982). Further, if all other variables are equal, Eqn. 5 shows that the
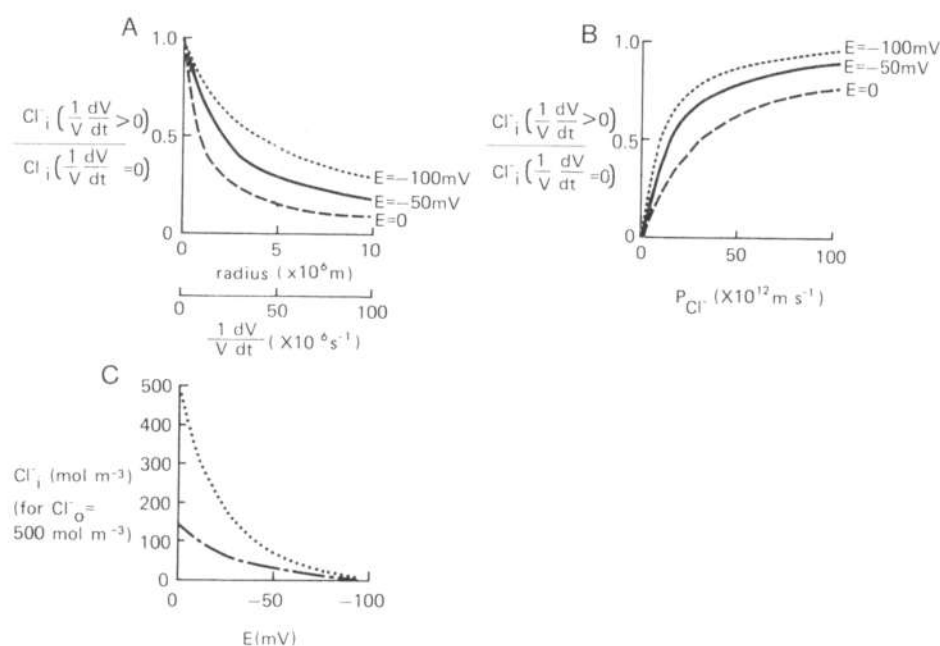
Figure 1. Effect of growth rate, cell radius, membrane permeability to Cl^-, and electrical potential on internal Cl^- concentrations. The figure is based on Eqn. 5 presented in the text (all Cl^- fluxes are assumed to be passive). (A) Effect of rate of volume expansion, or cell radius; (B) effect of P_{Cl^-}; (C) effect of E.

Parameters kept constant in particular cases were relative rate of volumetric expansion $= 30 \times 10^{-6}\ s^{-1}$, radius $= 3 \times 10^{-6}$ m [both values measured for *Chlorella emersonii* (Setter & Greenway, 1979)], and $P_{Cl^-} = 1 \times 10^{-11}\ m\ s^{-1}$. The ratio

$$\frac{[Cl^-]_i \text{ at a rate of volume expansion} > 0}{[Cl^-]_i \text{ at a rate of volume expansion} = 0}$$

is used to show the effect of volume expansion on $[Cl^-]_i$. (....) $[(1/V)\ (dV/dt)] = 0$; (— - —) $[(1/V)\ (dV/dt)] = 30 \times 10^{-6}\ s^{-1}$.

same dilution effect results from a smaller proportional expansion rate in cells with small rather than large surface area: volume ratio. This ratio differs substantially among species (Table 2).

The foregoing observations have assumed a particular value of P_{Cl^-}. Figure 1B shows that unless P_{Cl^-} is lower than about 50×10^{-12} m s^{-1} the influence of P_{Cl^-} on $[Cl^-]_i$ is not great. At higher permeabilities, the ratio of water flux to chloride flux is not high enough to keep $[Cl^-]_i$ much lower than $[Cl^-]_o$. (For this reason we took a P_{Cl^-} of 10×10^{-12} m s^{-1} for the demonstration of the effect

of rate of volume expansion, cell size, and E on internal Cl^- concentration; Fig. 1A, C.)

Figure 1C shows the dependence of $[Cl^-]_i$ on E. The electric field and dilution by water intake are the only factors which keep $[Cl^-]_i$ lower than $[Cl^-]_o$; therefore, one or both of E or $(1/A)\ (dV/dt)$ must be large to maintain low $[Cl^-]_i$, as Fig. 1C shows.

Equation 5 may also be used to indicate whether or not Cl^- fluxes in expanding cells are passive. This can be done in cases where both E and $[Cl^-]_i$ have been measured; in that case Eqn. 5 predicts whether E and $[Cl^-]_i$ are consistent with merely passive

Table 2. Area over volume ratio (A/V) and cell dimensions of some microalgae. The effect of changes in A/V on $[Cl^-]_i$ in growing cells is shown in Fig. 1A

Species	A/V (μm^{-1})	Cell dimensions (μm)	Reference
Aphanotece halophytica (grown in 2000 mol m^{-3} NaCl)	1.7	1×7 (ovaloid)	Miller *et al.* (1976)
Chlorella emersonii (grown in 335 mol m^{-3} NaCl)	1.0	6 (spherical)	Setter & Greenway (1979)
Platymonas suecica (grown in artificial seawater at 25 bar π_0)	0.17	9.5×12.5 (ovaloid)	Hellebust (1976)

fluxes. Consider the example of the freshwater species *Chlorella emersonii* grown at 335 mol m^{-3} NaCl (Greenway & Setter, 1979) in which [Cl$^-$]$_i$ was about 20 mol m^{-3}. The Nernst equation, which assumes no net water flux and therefore no growth, would predict an *E* of -70 mV. However, Eqn. 5 shows that for a relative growth rate of 3×10^{-5} s^{-1} (Greenway & Setter, 1979) and an assumed value for P_{Cl^-} of 10×10^{-12} m s^{-1} (as used for Fig. 1), *E* would be -50 mV. The electrical potential of *Chlorella emersonii* (or for any other *Chlorella* species) exposed to high (Cl$^-$]$_0$ has not been measured; however, an *E* of -50 mV is very similar to the value (*see above*) of *C. pyrenoidosa* and *C. fusca* grown in low [Cl$^-$]$_0$ measured with microelectrodes (Barber, 1968; Langmüller & Springer-Lederer, 1974). If this value is unchanged at high NaCl, then Cl$^-$ fluxes would be purely diffusive. If, however, these microelectrode measurements are artefactual and *E* is closer to the value of -135 mV measured by Komor & Tanner (1976) then this would suggest that there were substantial *active* Cl$^-$ influxes, even at this high external Cl$^-$ concentration. A similar inference can be made from data for the marine microalga *Platymonas subcordiformis*: [Cl$^-$]$_i$ was 70 mol m^{-3} when [Cl$^-$]$_0$ was 500 mol m^{-3} (Table 1).

Sodium fluxes in steady state

Our analysis of chloride fluxes assumed that, at high [Cl$^-$]$_0$, such fluxes were passive. However, an analysis of sodium fluxes must take into account the presence of an active 'pump' (a mechanism which causes a local flux against either concentration gradients or electric field or both while dissipating chemical energy). Because the electrical potential of most plant cells is negative, a low internal Na$^+$ concentration can be maintained at high [Na$^+$]$_0$ only when there is a large capacity for active Na$^+$ efflux. There is convincing evidence for energy-dependent Na$^+$ extrusion mechanisms in both the freshwater species *Chlorella pyrenoidosa* (Shieh & Barber, 1971) and *Chlorella fusca* (Tromballa, 1974) and in the marine species *Dunaliella tertiolecta* (Latorella & Vadas, 1973). The evidence favours Na$^+$/K$^+$ exchange for some species and Na$^+$/K$^+$ exchange mediated by proton fluxes in other species (Pitman & Lüttge, 1982). The type of Na$^+$ exchange mechanisms in marine microalgae have as yet not been established (Pitman & Lüttge, 1982), though there is some evidence for Na$^+$-amino acid co-transport in the direction of the electrochemical gradient for Na$^+$ (Raven, 1980).

In Table 3 we compare some measurements of the activity of Na$^+$ extrusion with likely rates of passive influx encountered by a microalgal cell at moderate salinity. Observed rates of Na$^+$ extrusion in the freshwater species *Chlorella pyrenoidosa* are in the order of 10 times greater than the passive Na$^+$ influx

Table 3. Comparison between calculated passive Na$^+$ influxes at high external Na$^+$ and observed rates of Na$^+$ extrusion from 'Na$^+$-rich' cells of *Chlorella pyrenoidosa*[*]
(a) Calculated passive influx for a gradient of 100 mol m^{-3} Na$^+$ across the plasmalemma[†]

E (mV)	Flux (mol m^{-2} s^{-1})
0	2×10^{-9}
-100	8×10^{-9}

(b) Observed rates of Na$^+$ extrusion

Na$^+$/Na$^+$ exchange	50–60×10^{-9}	Barber & Shieh (1973)
Na$^+$/K$^+$ exchange[‡]	150–200×10^{-9}	Shieh & Barber (1971), Barber & Shieh (1972)

[*]These cells were grown in 4–7 mol m^{-3} Na$^+$ and 0.04 mol m^{-3} K$^+$ and contained 55 mol m^{-3} Na$^+$ and 30 mol m^{-3} K$^+$.
[†] The value for P_{Na^+} assumed for this calculation was 2×10^{-11} m s^{-1}, estimated from the measured Na$^+$ influx (Shieh & Barber, 1971) and an *E* of -40 mV (Barber, 1968) for *Chlorella pyrenoidosa* measured at low external Cl$^-$. However, it is possible that *E* might be as low as -135 mV (cf. Komor & Tanner, 1976), thus giving a lower estimate for P_{Na^+}.
[‡] Na$^+$/K$^+$ exchange after 3 mol m^{-3} K$^+$ was added to nutrient.

calculated for a Na$^+$ gradient of 100 mol m^{-3} across the plasmalemma (Table 3). This indicates that the active Na$^+$ extrusion is sufficient to maintain relatively low internal Na$^+$ concentrations not only in freshwater species growing in 100 mol m^{-3} salt, but also in marine or salt lake species growing in much higher external salt concentrations where the gradient of [Na$^+$] can be very large, e.g. in *Dunaliella* (Table 1). For these calculations we used a permeability coefficient for Na$^+$ of 2×10^{-11} m s^{-1}. If, however, the permeability coefficient increases with increasing salinity, or is higher in other species of microalgae, then the greater diffusive Na$^+$ flux must be opposed by a greater active flux. A high P_{Na^-} certainly occurs in several macroalgae, the P_{Na^-} of both the tonoplast and plasmalemma being 3–1000 times higher than the plasmalemma of *Chlorella pyrenoidosa* (Raven, 1976).

A high growth rate is therefore much less likely to be of major importance in the regulation of [Na$^+$]$_i$ than of [Cl$^-$]$_i$ since the Na$^+$ extrusion pump probably has sufficient capacity to keep Na$^+$ low under most conditions. However, under certain conditions, cell expansion may be a significant factor.

In the case of Na$^+$, Eqn. 2 is replaced by

$$J = -\frac{zFE}{RT} \cdot P_x \frac{[a_0 - a_i \exp(zFE/RT)]}{1 - \exp(zFE/RT)} + J_p(a_0, a_i, E, T)$$

(6)

when J_p, the component of flux contributed by the pump, is a function of *E*, a_0, a_i, and T, which is not explicitly known (though this knowledge, of course,

is one of the objectives of plant electro-physiology).
Taking $z = 1$, and

$$\frac{1}{A} \cdot \frac{dV}{dt} = \frac{V}{A} \cdot G,$$

then Eqns 3 and 6 give

$$[Na^+]_i = \frac{[Na^+]_0 \exp(-FE/RT) + J_p \left(\frac{1}{P_{Na^+}}\right) \frac{1}{FE/RT} [1 - \exp(-FE/RT)]}{1 + \left(\frac{1}{P_{Na^+}} \cdot \frac{V}{A} \cdot G\right) \frac{1}{FE/RT} [1 - \exp(-FE/RT)]}. \quad (7)$$

Our ignorance of the analytic form of J_p renders Eqn. 7 less useful than Eqn. 5. It is likely that $[Na^+]_0$ and $[Na^+]_i$ and E affect J_p via mass action and activation energy, respectively. Further, the effects of $[Na^+]_i$ and E may be great if the pump may be described as a regulator of either variable.

If the pump was saturated under some conditions then one could use Eqn. 7 with constant J_p to examine the dependence of $[Na^+]_i$ on G. Under conditions which do not saturate the pump, it seems likely that changes in any variable will cause large changes in J_p. Nevertheless, Eqn. 7 can supply one useful relation: that between J_p and $(V/A) \cdot G$, with all other variables constant. That is, we may compare the active extrusion rates necessary to maintain identical E, $[Na^+]_0$, and $[Na^+]_i$ in two cells with different $(V/A) \cdot G$. This can be exemplified with some data for the salt lake species *Dunaliella parva*, taking $[Na^+]_i$ to be 700 mol m^{-3} at 1500 mol m^{-3} NaCl (*see* Table 1). In this species volume expansion due to growth would reduce the requirement for Na$^+$ extrusion by only 3–5%, though we remark that in some microalgae the volume expansion rate may be four times higher than in this example. (This value is calculated from measured values of 8×10^{-6} s^{-1} for the relative expansion rate, 2–3 μm for radius (Brüggemann *et al.*, 1978), and assumed values of 2×10^{-11} m s^{-1} for P_{Na+} and -100 mV for E.) A less negative E would, of course, reduce the requirement for Na$^+$ extrusion substantially. For example, an increase of E from -100 to -50 mV would reduce the Na$^+$ influx by 40%. A less negative E would increase the Cl$^-$ influx, but this would not necessarily lead to excessively high Cl$_i^-$ in rapidly growing cells (Fig. 1C).

The energy requirement for osmotic regulation and ion extrusion

A low cytoplasmic ion concentration means not only that energy may be expended in ion extrusion, but also that enough organic solutes must be synthesized to maintain volume in wall-less cells and turgor in walled cells. Energy requirements for ion extrusion and osmotic regulation could in principle be determined experimentally by distinguishing maintenance respiration from growth respiration, by such

procedures as discussed by Penning de Vries (1975). However, there are no suitable data available for algae growing at high salinity. We therefore consider the total energy requirements for osmotic regulation of a growing cell, using the example of *Dunaliella parva*, a species native to salt lakes. The $[Na^+]_i$ in this species is usually substantially lower than in the external medium (*see* Table 1), and we take here the case of $[Na^+]_i = 300$ mol m^{-3} at an external [NaCl] of 750 mol m^{-3} (Gimmler & Schirling, 1978). This partial exclusion of Na$^+$ adds a requirement for accumulation of other osmotic solutes, and glycerol fulfills most of this requirement in *D. parva* (Ben Amotz, 1975). The total cost of the Na$^+$ extrusion and glycerol accumulation equals about 10% of the carbohydrate consumed in the respiration measured at 750 mol m^{-3} NaCl (Table 4). Most of this cost is due to the requirement for accumulation of glycerol. The total energy requirement is rather modest, considering the high external salt concentration. Such a low energy expenditure, in terms of total respiration per gram protein, would be expected only in cells with small vacuoles. The cost of accumulation of organic solutes would be prohibitive in highly vacuolated cells; this point is taken up in the following paper.

The main cost of exclusion of ions is therefore the requirement for accumulation of organic osmotic solutes. This may constitute a diversion of carbohydrates from synthesis of other cell constituents, such as proteins and polysaccharides, into osmotic solutes. Nevertheless, this diversion is not excessive, at least for external NaCl concentrations as high as 750 mol m^{-3} and growth rates of 0.03 h^{-1} (Table 4). Many species of microalgae do grow faster than *Dunaliella parva*, but their growth rates seldom exceed 0.12 h^{-1} (Munns *et al.*, 1982). Thus, any limitations on growth of microalgae associated with the cost of osmotic regulation may occur only at very high salt concentrations found in salt lakes. This conclusion applies only to the cost of osmotic regulation in terms of total amount of carbohydrates required; other possible adverse effects of the accumulation of organic osmotic solutes are discussed elsewhere (Munns *et al.*, 1982).

Table 4. Energy requirements for a slightly vacuolated cell growing at high salinity*

	Energy requirement of cells growing at a relative rate of 0.03 h^{-1}	
	mmol O_2 g^{-1} (protein) h^{-1}	mg hexose g^{-1} (protein) h^{-1}
(i) Calculated respiration for Na^+ extrusion to maintain a 450 mol m^{-3} gradient between cell and external solution	0.082–0.127	2.5–3.8
(ii) Calculated respiration for synthesis of 450 mol m^{-3} glycerol	0.08	2.4
(iii) Calculated hexose requirement for synthesis of 450 mol m^{-3} glycerol	—	5.7
(iv) Measured rate of respiration (Gimmler; personal communication)	3.2	96

*The calculated energy requirements for Na^+ extrusion and synthesis of endogenous osmotic solutes, and observed rates of respiration, are compared for *Dunaliella parva* growing in 750 mol m^{-3} NaCl. Such cells contain about 300 mol m^{-3} Na^+, 50 mol m^{-3} K^+, and 450 mol m^{-3} glycerol as the major endogenous osmotic solutes (Gimmler & Schirling, 1978). The relative growth is about 0.03 h^{-1}, and the cell radius is 2–3 μm (Brüggemann *et al.*, 1978).

It is assumed that (i) the respiration of 1 mol of hexose consumes 6 mol of O_2 and yields 24 mol of ATP; (ii) the synthesis of 1 mol of glycerol from one hexose unit (starch) requires 2 mol of NADPH and 1 mol of ATP (Kaplan *et al.*, 1980); (iii) the hydrolysis of 1 mol of ATP yields 33 kJ; (iv) P_{Na^+} is 2×10^{-11} m s^{-1}; (v) E is -100 mV; (vi) cell water : protein ratio is 3.5 : 1; and (vii) the energy required to extrude Na^+ is the difference in free energy per mol (given, e.g., by Lüttge & Pitman, 1976) times the active flux (Eqn. 6).

Conclusions

In slightly vacuolated cells the concentrations of Cl^- and Na^+ are lowered by a combination of rapid volume expansion and Na^+ extrusion. Thus, adverse effects of Cl^- and Na^+ on metabolism are avoided at the very time that high rates of metabolism are required. The converse is that $[Cl^-]_i$ and $[Na^+]_i$ may be considerably higher during slow than during rapid growth. Perhaps slowly growing cells have mechanisms to lower $[Cl^-]_i$ and $[Na^+]_i$ before resuming rapid growth. For example, a rapid Cl^- efflux could occur if E became temporarily much more negative and this would give merely a transient increase in the energy required for Na^+ extrusion during the initiation of rapid cell expansion. Nevertheless, during steady-state growth, the energy required for ion extrusion is minimal, and the carbohydrate used for osmotic regulation is still only a small proportion of the total consumed in respiration. So, to explain the reduction in growth suffered by many microalgae at high salinity we must consider factors other than energy requirements, such as adverse effects caused (directly or indirectly) by high concentrations of organic solutes.

Acknowledgments

Appendix

Abbreviations

A	surface area;
a	activity of a solute;
E	electrical potential difference between cell and external solution;
F	Faraday (at 25°C, $F/RT = 38.9$ V^{-1});
G	relative rate of expansion of volume or cell water $[(1 \cdot dV)/(V\,dt)]$;
P_X	permeability of membrane to solute X;
R	gas constant;
r	radius;
T	temperature, Kelvin;
t	time;
V	volume of water inside the plasmalema;
$[X]$	concentration of solute X;
z	electronic charge of ion;
subscript i	inside the cell membrane
subscript 0	outside the cell membrane.

Definitions

Active ion flux. Energy-dependent movement of ions against an electrochemical potential gradient via porters (carriers).

Passive ion flux. Movement of ions across a membrane along an electrochemical potential gradient. This could be via
(i) porters (carriers), which could be specific to a particular ion.

(This flux is also called *facilitated* or *carrier-mediated* diffusion.)

(ii) Ion channels (leak channels), which are usually non-specific.

(iii) Pores in the lipid bilayer. (This flux is very small in comparison to (i) and (ii).)

Osmotic regulation. Metabolic processes resulting in changes in internal solute contents which lead to turgor or volume maintenance.

References

Barber, J. (1968) Measurement of the membrane potential and evidence for active transport of ions in *Chlorella pyrenoidosa*. *Biochimica et Biophysica Acta*, **150**, 618–625.

Barber, J. & Shieh, Y.J. (1972) Net and steady-state cation fluxes in *Chlorella pyrenoidosa*. *Journal of Experimental Botany*, **23**, 627–636.

Barber, J. & Shieh, Y.J. (1973) Sodium transport in Na$^+$ rich *Chlorella* cells. *Planta*, **111**, 13–22.

Ben-Amotz, A. (1975) Adaptation of the unicellular alga *Dunaliella parva* to saline environment. *Journal of Phycology*, **11**, 50–54.

Briggs, G.E., Hope, A.B. & Robertson, R.N. (1961) Electrolytes and plant cells. In *Botanical Monographs* (ed. W.O. James), Vol. 1. Blackwell Scientific Publications, Oxford.

Brüggemann, M., Weiger, C. & Gimmler, H. (1978) Synchronized culture of the halotolerant unicellular green alga *Dunaliella parva*. *Biochemie und Physiologie der Pflanzen*, **172**, 487–506.

Gimmler, H. & Schirling, R. (1978) Cation permeability of the plasmalemma of the halotolerant alga *Dunaliella parva*. II. Cation content and glycerol concentration of the cells as dependent upon extracellular NaCl concentration. *Zeitschrift für Pflanzenphysiologie*, **87**, 435–444.

Greenway, H. & Munns, R. (1980) Mechanisms of salt tolerance in non-halophytes. *Annual Reviews of Plant Physiology*, **31**, 149–190.

Greenway, H. & Setter, T.L. (1979) Na$^+$, Cl$^-$ and K$^+$ concentrations in *Chlorella emersonii* exposed to 100 and 335 mM NaCl. *Australian Journal of Plant Physiology*, **6**, 61–67, and corrigendum in *Australian Journal of Plant Physiology*, **6**, 569–572.

Hellebust, J.A. (1976) Effect of salinity on photosynthesis and mannitol synthesis in the green flagellate *Platymonas suecica*. *Canadian Journal of Botany*, **54**, 1735–1741.

Jennings, D.H. (1969) The physiology of the uptake of ions by the growing plant cell. In *Ecological Aspects of the Mineral Nutrition of Plants* (ed. I.H. Rorison), pp. 261–279. Blackwell Scientific Publications, Oxford.

Jennings, D.H. (1976) The effects of sodium chloride on higher plants. *Biological Reviews*, **51**, 453–486.

Kaplan, A., Schreiber, U. & Avron, M. (1980) Salt induced changes in *Dunaliella salina*. *Plant Physiology*, **65**, 810–813.

Kauss, H. (1978) Osmotic regulation in algae. *Progress in Phytochemistry*, **5**, 1–27.

Kirst, G.O. (1977a) Ion composition of unicellular marine and freshwater algae, with special reference to *Platymonas subcordiformis* cultivated in media with different osmotic strengths. *Oecologia*, **28**, 177–189.

Kirst, G.O. (1977b) The cell volume of the unicellular alga *Platymonas subcordiformis* Hazen: Effect of the salinity of the culture media and of osmotic stresses. *Zeitschrift für Pflanzenphysiologie*, **81**, 386–394.

Komor, E. & Tanner, W. (1976) The determination of membrane potential of *Chlorella vulgaris*. Evidence for electrogenic sugar transport. *European Journal of Biochemistry*, **70**, 197–204.

Langmüller, G. & Springer-Lederer, H. (1974) Dependence of membrane potential of *Chlorella fusca* on pH, temperature and light. *Planta*, **120**, 189–196.

Latorella, A.H. & Vadas, R.L. (1973) Salinity adaptation by *Dunaliella tertiolecta*. I. Increases in carbonic anhydrase activity and evidence for a light-dependent Na$^+$/H$^+$ exchange. *Journal of Phycology*, **9**, 273–277.

Levring, T., Hope, H.A. & Schmid, O.J. (1969) Marine algae: A survey of research and utilization. In *Botanica Marine Handbooks*, Vol. 1. Cram de Gruyter & Co., Hamburg.

Lüttge, U. & Pitman, M.G. (1976) Transport and energy. In *Transport in Plants II. Part A, Cells. Encyclopedia of Plant Physiology* New Series Volume 2 (eds U. Lüttge & M.G. Pitman), pp. 251–259. Springer-Verlag, Berlin, Heidelberg, New York.

Miller, D.M. Jones, J.H., Yopp, J.H., Tindall, D.R. & Schmid, W.E. (1976) Ion metabolism in a halophilic blue green alga, *Aphanothece halophytica*. *Archives of Microbiology*, **III**, 145–159.

Munns, R., Greenway, H. & Kirst, G.O. (1982) Halophytes; halotolerant eukaryotes. In *Encyclopedia of Plant Physiology* (eds O.L. Lange, C.B. Osmond, P.S. Nobel & H. Ziegler), Vol. 12C. Springer-Verlag, Berlin, Heidelberg, New York (in press).

Penning de Vries, F.W.T. (1975) The cost of maintenance processes in plant cells. *Annals of Botany*, **39**, 77–92.

Pitman, M.G. & Lüttge, U. (1982) The ionic environment and plant ionic relations. In *Physiological Plant Ecology. Encyclopedia of Plant Physiology* Vol. 12C (eds O.L. Lange, C.B. Osmond, P.S. Nobel & H. Ziegler). Springer-Verlag, Berlin, Heidelberg, New York (in press).

Raven, J.A. (1976) Transport in algal cells. In *Encyclopedia of Plant Physiology* New Series Volume 2 (eds U. Lüttge & M.G. Pitman), Chap. 6, Part A, pp. 129–188. Springer-Verlag, Berlin, Heidelberg, New York.

Raven, J.A. (1980) Nutrient transport in microalgae. *Advances in Microbial Physiology*, **21**, 47–226.

Setter, T.L. & Greenway, H. (1979) Growth and osmoregulation of *Chlorella emersonii* in NaCl and neutral osmotica. *Australian Journal of Plant Physiology*, **6**, 47–60, and corrigendum in *Australian Journal of Plant Physiology*, **6**, 569–572.

Shieh, Y.J. & Barber, J. (1971) Intracellular sodium and potassium concentrations and net cation movements in *Chlorella pyrenoidosa*. *Biochimica et Biophysica Acta*, **223**, 594–603.

Tromballa, H.W. (1974) Influence of pH on uptake and release of sodium of *Chlorella*. *Planta*, **117**, 339–348.

Wyn Jones, R.G., Brady, C.J. & Speirs, J. (1979) Ionic and osmotic relations in plant cells. In *Recent Advances in the Biochemistry of Cereals* (ed. D.L. Laidman & R.G. Wyn Jones), pp. 63–104. Academic Press, New York, London.