BBA 75320

IONIC FLUXES IN CELLS OF *CHARA CORALLINA*

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(Received March 31st, 1969)

SUMMARY

The influx and efflux of Cl^-, K^+ and Na^+ have been measured in a series of experiments with comparable cells of *Chara corallina*, under different experimental conditions.

Several "states" of Chara cells have been found. In the state which is most often found, Cl^- influx is light-stimulated and requires K^+ or Na^+, and K^+ influx is light-stimulated and dependent on Cl^- in the medium. These fractions of the Cl^- and K^+ influx are about 1 pmole·cm^{-2}·sec^{-1} on the average. Other states have been found in which neither Cl^- nor K^+ influx is light-stimulated, or in which Cl^- influx is light- and K^+-dependent while K^+ influx is light- but not Cl^--sensitive.

A residual Cl^- influx in the dark is postulated to be part of an exchange system with an equal efflux of Cl^-, and not active transport.

Neither K^+ influx nor Na^+ efflux were sensitive to up to 1 mM ouabain in the medium, in contrast to reports that cells of *Nitella translucens* and *Hydrodictyon africanum* are ouabain-sensitive.

A proposed passive component of the K^+ influx was sometimes, but not always, consistent with observed changes in the potential difference and conductance of the plasmalemma.

INTRODUCTION

MACROBBIE[1-3] has shown that two major ion-transport systems control the movement of ions into and out of *Nitella translucens*. The operation of an inward Cl^- pump, accompanied by cation flow, is responsible for the accumulation of KCl and NaCl by the cells. The cation content is further regulated by the coupled active transport of K^+ inwards and Na^+ outwards. The influxes of K^+, Na^+ and Cl^- are greatly increased by light and MACROBBIE[1-3] has concluded that the Cl^- pump is powered by photosynthetic electron flow while the K^+ pump requires ATP. This was suggested by the differential effects of uncouplers of photophosphorylation, on the one hand, and of far red light and the inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) on the other. Uncouplers tended to reduce K^+ influx, leaving that of

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; FPW, artificial pond water containing NaCl, 2 mM; KCl, 0.2 mM; $CaCl_2$, 0.05 mM.

Cl^- high, while far red light and DCMU reduced the Cl^- influx but left K^+ influx appreciable. Results similar to those of MACROBBIE[1-3] have been obtained by RAVEN[4-6] with *Hydrodictyon africanum*.

Investigations of the ionic relations of *Chara corallina*[7-9] revealed important similarities to those of *N. translucens* and *H. africanum*. For example, in *C. corallina* transport of Cl^- inwards and Na^+ outwards is increased by light while effects of light on passive Na^+ influx were reported by HOPE AND WALKER[8]. K^+ uptake is apparently wholly passive. COSTER AND HOPE[7] and SMITH AND WEST[9] reported some effects of inhibitors on ion fluxes in *C. corallina* which did not appear to be compatible with MacRobbie's hypothesis.

The present paper reports a further detailed examination of the ionic relations of *C. corallina*. The work had several major aims. The first was to draw up a comprehensive 'balance sheet' of ion fluxes into and out of similar cells. Although previous work with *H. africanum*[6] and *N. translucens*[10] suggested that there are links between passive cation uptake and the Cl^- pump, the nature of the links remained in doubt. In the present study, special attention was paid to measurements of K^+ and Cl^- fluxes using double labelling of cells with $^{42}K^+$ and $^{36}Cl^-$. As there is reason to believe that the influx of K^+ is under the influence of the electrochemical potential gradient at the plasmalemma and of the permeability of this membrane[11], the electrical properties of the cells under the various experimental conditions were also measured.

The effects of metabolic inhibitors have also been reexamined, with measurements of ion fluxes, electrical properties and also of metabolic processes in the cells. These results will be discussed in a subsequent paper.

MATERIAL AND METHODS

Cells of *Chara corallina* (formerly *Chara australis*) R.Br., cultured in tanks in Flinders University, were used throughout. The plants originally came from Ross, Tasmania. Cells were trimmed of whorl (leaf) cells and placed in an artificial pond water (FPW) comprising NaCl, 2 mM; KCl, 0.2 mM; $CaCl_2$, 0.05 mM, for 24 h in the light, before use in experiments to measure flux or electrical characteristics. Batches of 7–10 cells were used for a determination of mean flux under a particular condition.

$^{36}Cl^-$ as NaCl and $^{22}Na^+$ as NaCl were supplied by the Radiochemical Company (Amersham, U.K.) at high specific activity. $^{42}K^+$ in the form of K_2SO_4 (enriched with $^{41}K^+$) and $^{24}Na^+$ as NaCl were supplied by the Australian Atomic Energy Commission; specific activity on arrival was usually greater than 1 mC/mg salt.

Simultaneous determination of influx of any two of K^+, Na^+, Cl^- was done by adding appropriate amounts of one long-lived and one short-lived isotope and counting cells and aliquots of the test medium twice with an interval of several days. A digital computer programme was then used to calculate fluxes from this data and that for cell dimensions, ion concentration, *etc.* In the text, the mean flux is given, together with the standard error of the mean, and the number of cells in brackets.

Techniques for measuring the vacuolar potential difference with respect to the medium, and the electric resistance of the plasmalemma, have been described previously[12].

The main experimental solution was FPW (above). When Cl^--free solutions

were required, sodium, potassium and calcium sulphates were used. When K^+-free, or K^+, Na^+-free solutions were called for, choline chloride or tetra-ethylammonium chloride was used to make up the medium. Analytical grade chemicals were used. Pretreatment consisted of rinsing cells twice with the altered solution and soaking for a further 2 h. Thus in "Cl^--free" or "K^+-free" solutions the only significant source of these ions would have been the cells themselves. The pH of the solutions was 5.5–5.8.

Ouabain was obtained from Sigma Chemical Company and checked for inhibiting ability on K^+/Na^+ transport in an animal preparation.

RESULTS

Cl^- influx

Dark conditions. Table I lists several experiments in which dark and other conditions were tested. Expt. 1 was an exception in which the effect of dark was not significant. In most experiments there was a prompt decrease in influx upon darkening. A pretreatment dark time of up to 17 h was used in many experiments; however, the dark decrease was later established as occurring within the first 2 h of darkness.

Removal of K^+ and Na^+. In Table I, 4 batches of cells out of 5 showed a dependence of Cl^- influx on K^+ in the medium. Expt. 1 was again an exception. In Expts. 1, 2 and 4, Na^+ may have supported Cl^- influx. However, when measured (Expt. 2), Na^+ influx did not increase over control values enough to match the absent K^+ influx.

Influx under CO_2-free conditions. In the experiments shown in Table II, N_2 or O_2 was bubbled during the 1.5–2-h pretreatment, and during the period of radioactive solution. Even under these CO_2-free conditions the Cl^- influx was higher in the light than in the dark. It is interesting that the dark influx did not appear to depend on the presence of O_2.

TABLE I

INFLUXES OF Cl^- AND K^+ UNDER VARIOUS CONDITIONS

The units of influx are pmole·cm^{-2}·sec^{-1}.

Expt. No.	*Influx of*	*Control*	*Dark*	*Dark, −Cl⁻*	*−K⁺*	*−Cl⁻*
1	Cl^-	1.41 ± 0.19 (8)	0.98 ± 0.22 (9)	—	1.50 ± 0.24 (8)	—
	K^+	1.11 ± 0.083 (16)	1.39 ± 0.14 (25)	1.71 ± 0.42 (8)	—	0.99 ± 0.13 (9)
2	Cl^-	1.56 ± 0.15 (17)	0.39 ± 0.14 (9)	—	0.73 ± 0.10 (9)	—
	K^+	1.35 ± 0.15 (17)	0.14 ± 0.02 (17)	—	—	0.68 ± 0.05 (9)
3	Cl^-	1.65 ± 0.35 (10)	0.24 ± 0.051 (10)	—	0.39 ± 0.05 (10)*	—
	K^+	1.63 ± 0.37 (10)	0.11 ± 0.032 (10)	0.16 ± 0.08 (10)	—	1.36 ± 0.18 (10)
4	Cl^-	3.30 ± 0.50 (9)	—	—	0.87 ± 0.22 (9)	—
5	Cl^-	1.45 ± 0.21 (9)	—	—	0.53 ± 0.22 (9)*	—
6	Cl^-	1.49 ± 0.12 (10)	0.58 ± 0.09 (10)	—	—	—
	K^+	1.26 ± 0.20 (10)	0.26 ± 0.040 (10)	—	—	—

* Na^+ omitted as well as K^+.

TABLE II

EFFECT ON Cl^- INFLUX OF N_2 REPLACING O_2 AND CO_2 IN MEDIUM

Expt. No.	*Cl^- influx* (*pmole·cm^{-2}·sec^{-1}*)	*Conditions*	*Influx* (*pmole·cm^{-2}·sec^{-1}*)	*Conditions*
6	1.49 ± 0.12 (10)	Light, stagnant	0.97 ± 0.10 (10)	Light, N_2-bubbled
7	1.98 ± 0.34 (7)	Light, O_2-bubbled	0.75 ± 0.06 (7)	Light, N_2-bubbled
7	0.20 ± 0.055 (7)	Dark, O_2-bubbled	0.16 ± 0.017 (7)	Dark, N_2-bubbled

TABLE III

MEAN Cl^- EFFLUXES IN pmole·cm^{-2}·sec^{-1} IN 4–5½-h PERIODS OF ELUTION

Time after removal from labelling solution (*h*)	*Treatment*	*Cell:* 1	2	3
20–27	Light, FPW	0.51	0.54	0.46
	Light, $-Cl^-$, $+SO_4^{2-}$	0.14	0.10	0.082
	Light, FPW	0.38	0.46	0.41
41–48	Light, FPW	0.31	0.45	0.26
	Light, $-Cl^-$, $+Br^-$	0.35	0.60	0.31
	Light, FPW	0.38	0.55	0.25
	(Dark interval between 48 and 65 h)			
65–72	Dark, FPW	0.95	0.73	0.83
	Dark, $-Cl^-$, $+SO_4^{2-}$	0.51	0.35	0.46
	Dark, FPW	0.82	0.65	1.12
89–96	Dark, FPW	0.43	0.71	0.69
	Dark, $-Cl^-$, $+Br^-$	0.39	0.66	0.54
	Dark, FPW	0.43	0.67	0.42

Cl^- efflux

When labelled cells were eluted with inactive FPW, the apparent rate constant for exchange of the internal Cl^- across the plasmalemma (the limiting step) was found to decrease over about 24 h, and then become nearly constant. Table III lists the efflux under various conditions, calculated from the vacuolar specific activity, which was measured at the end of the experiment. This flux corresponds to ψ_{co}, the efflux across the plasmalemma, according to the following reasoning. The plasmalemma has been shown to be rate-limiting for influx[1,7], the influx at the tonoplast being 30–200 pmoles·cm^{-2}·sec^{-1}. Unless the efflux across the tonoplast is almost as large, the cytoplasm would become quickly depleted of Cl^-. If the efflux at the tonoplast is of the order of 50 pmoles·cm^{-2}·sec^{-1}, the observed rate constant leads to a value of the order of 1 pmole·cm^{-2}·sec^{-1} for ψ_{co}.

Table III shows that the Cl^- efflux rose in the dark and that when external Cl^- was replaced by SO_4^{2-} (either in light or dark) the Cl^- efflux was substantially decreased, confirming earlier reports[13]. However, when Cl^- was replaced by Br^- there was no reduction.

K^+ fluxes and electrical measurements

Dark conditions. In media containing either Cl^- or SO_4^{2-} as the anion, dark conditions usually reduced the K^+ influx. Representative experiments are shown in Table I. The dark reduction of influx generally found was correlated with sensitivity of the Cl^- influx to dark; insensitivity was found in the same batch of cells in which Cl^- influx was light/dark insensitive (Expt. 1). When dark reduction occurred, it did so within 2 h of darkening, as with Cl^-.

In view of the possible contribution of passive fluxes, and changes in these due to changes in membrane properties in the dark, the effect of darkening on potential difference and resistance was investigated. It was previously reported that in Cl^- media the effect of light/dark changes was small in Chara[14]. The electrical measurements were made on cells of the same batch as used in Expt. 3, Table I.

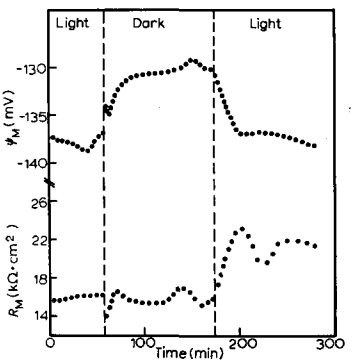

Fig. 1. The potential difference, ψ_M, and the resistance, R_M, between the vacuole of a cell of *C. corallina* and a stagnant, external medium of FPW. At the times indicated, the preparation was in light or dark. In Figs. 1, 3 and 4, the points are values taken from a continuous chart recording of ψ_M and frequent recordings of R_M.

A consistent depolarization of 7–10 mV was observed less than 1 h after darkening, in the present experiments. The time course of potential difference and resistance in a sample experiment is shown in Fig. 1. The changes in resistance tended to be oscillatory in nature, and not as consistent as the change in potential difference. Both the resistance and potential difference are that of the plasmalemma and tonoplast in series.

Removal of Cl^-. Table I also shows that when SO_4^{2-} replaced Cl^- in the medium, K^+ influx was reduced in one experiment (Expt. 2) but almost unaffected in two others (Expts. 1 and 3). It is also to be noted that in Expt. 3, the influx was lower in the dark in the absence of Cl^- as well as in its presence.

Fig. 2 shows the results from a large number of cells in which Cl^- and K^+ influxes were measured simultaneously. In two of the three series of experiments shown (2a and 2b) there was good correlation between the fluxes, but in the third series (Fig. 2c) the correlation was less marked.

Within 30 min of replacing Cl^- by SO_4^{2-} in the medium, the potential difference became depolarized to a new steady level, in five out of six experiments. Concurrently, the resistance dropped to 60 % of the level in Cl^--FPW. These effects were reversible. The mean depolarization, taken as the difference between the levels in SO_4^{2-}-FPW

and the mean levels before and after Cl^--FPW, was 14 ± 1.6 mV (5). The mean drop in resistance, calculated in the same way, was 6.0 ± 0.04 kΩ·cm² (5) from 15 kΩ·cm². Fig. 3 shows one of these experiments.

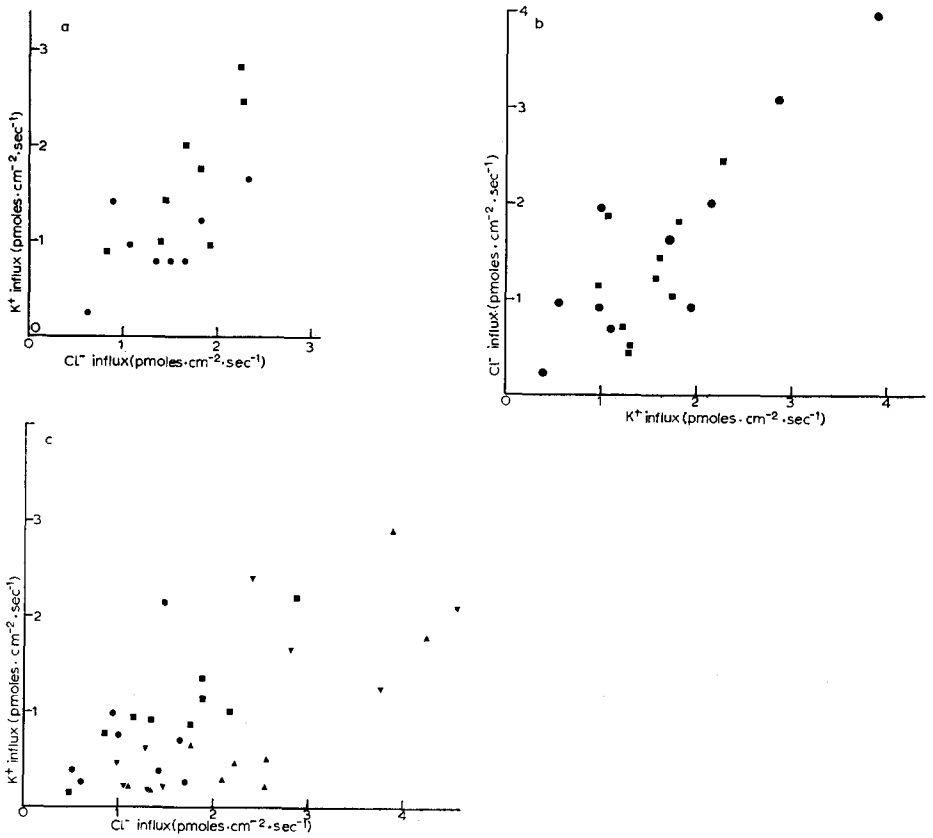

Fig. 2. The relation between Cl^- influx and K^+ influx when measured simultaneously in individual cells. (a), (b) and (c) represent difference batches of cells harvested weeks or months apart. Within the batches the different symbols represent experiments with 7–10 cells. The cells were in FPW in the light.

Effect of ouabain. MACROBBIE[1] and RAVEN[4] have reported inhibitory effects of 10^{-4}–10^{-3} M ouabain on K^+ influx and Na^+ efflux in Nitella and Hydrodictyon. It was concluded that a coupled K^+/Na^+ pump, similar (in its coupling) to that found in animal cells, may operate in these plant cells. In the present series of experiments, no consistent effect was noted at concentrations up to 1 mM (Table IV).

Effect of bubbling N_2. The influx of K^+ was reduced from a control value of 1.26 ± 0.20 (10) (Expt. 6) to 0.26 ± 0.051 (10) when N_2 gas (O_2-free) was bubbled through the pretreating and the active solutions for a total time of 3–4 h.

The potential difference and resistance were measured in several other cells in FPW which was continuously bubbled with N_2 before being flowed quickly past the cells. A hyperpolarization from about −145 to −190 mV was caused by the N_2 treatment. This was reversed on returning to FPW in equilibrium with air. There was

little effect on the cell resistance. The effect of N_2 was prevented when a gas mixture of N_2 + 0.1 % CO_2 was used. Fig. 4 illustrates the effects of N_2 alone, and of $N_2 + CO_2$. The pH of these solutions was 5.7.

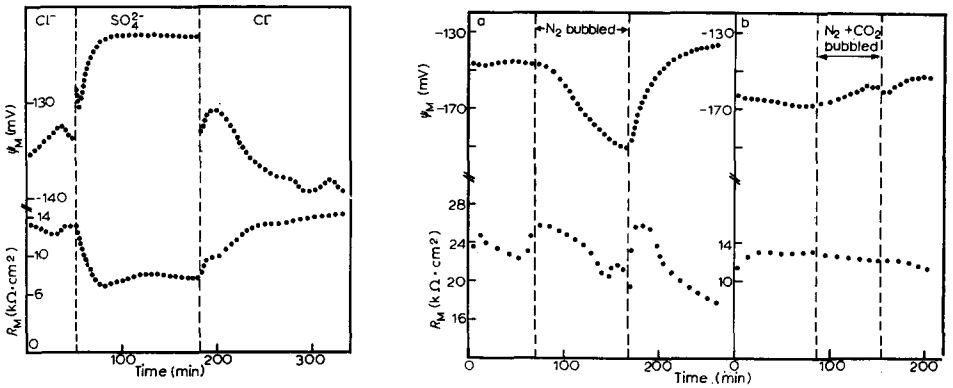

Fig. 3. The potential difference, ψ_M, and resistance, R_M, between the vacuole and external FPW or SO_4^{2-}-FPW, plotted against time, with solution changes where indicated.

Fig. 4. (a) The effect of bubbling N_2 through a reservoir of FPW solution subsequently flowed past a cell from which ψ_M and R_M were recorded. FPW in equilibrium with air was the medium before and after the N_2-bubbled solution. (b) As for (a) but bubbling with a gas mixture of N_2 + 0.1 % CO_2.

TABLE IV

EFFECT OF OUABAIN ON K^+ INFLUX

Expt. No.	*Control influx* (*pmole·cm⁻²·sec⁻¹*)	*Influx with ouabain*	*Ouabain concn.* (*mM*)
1	1.11 ± 0.083 (16)	1.65 ± 0.16 (8)*	0.3
8	1.11 ± 0.10 (17)	0.73 ± 0.16 (7)**	0.3
9	0.77 ± 0.20 (9)	1.03 ± 0.18 (9)	1.0
10	0.49 ± 0.10 (9)	0.55 ± 0.15 (8)	1.0
11	0.59 ± 0.09 (10)	0.89 ± 0.19 (9)	1.0

* Significant stimulation.
** Marginally significant inhibition. Other experiments, no significant effect.

K^+ efflux. This was not studied as systematically as the influx. The average efflux, measured after 8–10 h of elution to exchange the cytoplasmic activity, was about 0.6 pmole·cm^{-2}·sec^{-1}, from cells similar to those used in Expt. 3.

Cytoplasm rate constant. The relative amounts of radioactivity in the vacuole and cytoplasm after short labelling times were used to estimate the rate constant for exchange of cytoplasmic K^+, and the influx across the plasmalemma. The method has been described previously[1,7], for estimating these quantities for Cl^- ions. From the rate constant (k_c) and total amount of K^+ in the cytoplasm (Q_c) the flux from cytoplasm to vacuole (ψ_{cv}) can be deduced. The following table summarizes results and calculations from two batches of cells:

ψ_{oc} ($pmole \cdot cm^{-2} \cdot sec^{-1}$)	k_c (sec^{-1})	Q_c ($nmole \cdot cm^{-2}$)	$\psi_{co} + \psi_{cv}$ ($pmole \cdot cm^{-2} \cdot sec^{-1}$)	*Inferred* ψ_{cv}
0.37 ± 0.12 (9)	1.5 ± 0.2 · 10^{-3} (9)	(130)	195	195
1.01 ± 0.11 (9)	1.06 ± 0.13 (9)		140	140

The quantity in column 4, the sum of fluxes out of the cytoplasm, $\psi_{co} + \psi_{cv} = k_c \cdot Q_c$ (see refs. 1 and 7). (ψ_{oc} is the flux from the medium to the cytoplasm and ψ_{co} that in the opposite direction.) The figure for Q_c is from unpublished data of A. B. HOPE obtained using a perfusion technique (see ref. 7).

Na^+ fluxes

The usual level of Na^+ influx in the light from FPW medium was 0.2–0.5 pmole · cm^{-2} · sec^{-1}. Effects on Na^+ influx of darkness and of removal of K^+ from the medium were also noted. The influx in the dark was generally reduced. In the light, the absence of K^+ increased the Na^+ influx marginally. The efflux, after the cells had been loaded with tracer for 3–5 days, was generally rather larger than the influx, measured as an average over that time. Preparations which were loaded for efflux measurements by immersing the whole "cell", including small nodal cells at each end, gave an erroneously high efflux, for at least a day after elution began. Such cells were therefore eluted for about one day before collections were made for the estimation of efflux. Table V lists the effluxes found under various conditions. It is seen that ouabain up to 1 mM, and the absence of K^+ in the external medium had no noticeable effect on Na^+ efflux.

TABLE V

Na^+ FLUXES UNDER VARIOUS CONDITIONS

	Cell:	*1*	*2*	*3*	
Influx		0.25	0.25	0.23	Nodes in labelling solution
Efflux (3–5 h)		1.3	2.1	1.4	during influxing period.
Efflux (27–29 h)		0.80	0.71	0.78	
	Cell:	*4*	*5*	*6*	
Influx		0.50	0.21	0.35	Nodes in labelling solution,
Efflux, light		0.34	0.42	0.34	but pre-elution of 18 h.
light, +0.3 mM ouabain		0.33	0.43	—	
light, $-K^+$		—	—	0.34	
dark		0.21	0.61	0.38	Predarkening of 17 h.
dark, +ouabain		0.23	0.56	—	
dark, $-K^+$		—	—	0.36	
	Cell:	*7*	*8*	*9*	
Efflux, light		0.23	0.32	0.34	Pre-eluted 21 h.
light, +1 mM ouabain		0.21	0.37	—	
light, $-K^+$		—	—	0.36	

DISCUSSION

Cl^- influx

The results reported above are in general agreement with previous work on *C. corallina*. The Cl^- influx was normally stimulated by light and reduced when K^+ and Na^+ were removed. These effects were variable: in some experiments there was no significant stimulation by light, for example Table I, Expt. 1, where there was also little effect of removing K^+. It is to be noted that in this series the Cl^- influx in the dark was about 1 pmole·cm^{-2}·sec^{-1}, much greater than the normal dark value of 0.3–0.4 pmole·cm^{-2}·sec^{-1}. The K^+ influx was also normally increased by light and often reduced when Cl^- was removed. Again the magnitude of these effects varied.

Results obtained with Nitella[10], Hydrodictyon[6] and Tolypella[15] suggested that there is an association between cation and anion fluxes in the light (the cation-Cl^- system). Such an association appeared in many of our experiments, as is shown in Table I. Thus the light-stimulated component of Cl^- influx was very similar in size to the K^+/Na^+-dependent component.

The effects of N_2 on Cl^- influx are similar to those reported by MACROBBIE[3]. The partial inhibition in N_2 could be due to lack of either CO_2 or O_2. The lack of inhibition in pure O_2 shows that CO_2 is not required for high levels of Cl^- influx. The links between the Cl^- influx and photosynthesis will be discussed in more detail in a following paper.

The ratio of Cl^- efflux to influx in the dark approached 1.0. The value expected on the basis of passive, independent diffusion is of the order of 10[4]. This departure from passive, independent diffusion suggests that practically the whole of the influx in the dark is mediated by active transport or by exchange diffusion. The finding that removal of O_2 did not decrease the dark influx (Table II) indicates that any active influx must depend on glycolysis rather than respiration. Because of the reduction of Cl^- efflux (in light or darkness) in the absence of external Cl^- or Br^- it seems more likely that there is exchange diffusion with external Cl^- or Br^- partaking in an exchange with cytoplasmic Cl^-. It should be noted that SO_4^{2-} influx into *C. corallina* is very low, while the influx of Br^- has been found to be similar to that of Cl^- (N. A. WALKER, unpublished results).

The component of Cl^- efflux which depends on external Cl^- or Br^- is about 0.4 pmole·cm^{-2}·sec^{-1} in either light or dark. This is similar in size to the Cl^- influx remaining when the cells are in the dark, or in K^+, Na^+-free solutions. The data are thus consistent with an exchange component of about 0.4 pmole·cm^{-2}·sec^{-1} in the Cl^- effluxes. However, we do not know if in cells having a high dark influx (Expt. 1) the influx is independent of respiration. Any remaining Cl^- efflux is regarded as a passive, diffusive "leak" down the electrochemical gradient and under the influence of the membrane potential difference[7,13]. Its magnitude in the present series was about 0.1 pmole·cm^{-2}·sec^{-1} in light and up to 0.4 in the dark.

K^+ influx

Previous work on giant algal cells has suggested that the potassium influx may have the following components: (a) active, linked to Na^+ excretion by a pump sensitive to ouabain; (b) passive, under the influence of concentration, membrane potential, and permeability; (c) linked to Cl^- uptake by a pump connected to Photosystem 2.

Previous work with Chara[16] has shown no sign of component (a), and the present work confirms this. Ouabain in high concentration has shown no consistent inhibition of K^+ influx or Na^+ efflux, and the Na^+ efflux has been shown to be independent of the presence of external K^+.

In the absence of an active influx of type (a) we expect the K^+ influx in darkness or from SO_4^{2-} media to be passive *i.e.*, to represent component (b). On transfer to light this component may increase as is shown by higher K^+ influxes in light from SO_4^{2-} media (Table I, Expt. 3).

Factors which may lead to an increase in passive influx are a change to a more negative plasmalemma potential difference, or an increase in permeability to K^+. Such an increase was postulated for Nitella, Hydrodictyon and Tolypella, to account for the Cl^--independent cation influx in these species. Electrical measurements in SO_4^{2-} media, upon light to dark transitions, have not yet been made.

This work has shown that component (c) is usually present, but is very variable (a mean of 0–0.7 pmole·cm^{-2}·sec^{-1} in experiments tabled above; see also Fig. 2. Component (c) may in fact be a separate component carried by the Cl^- pump mechanism or a special case of component (b), in which Cl^- transport alters the effective membrane potential difference.

Electrical effects

BARRY AND HOPE[17] have shown theoretically that depletion of K^+ just outside the plasmalemma during the sustained passage of an inward electric current may cause a hyperpolarization which increases with time until depletion is balanced by diffusion through the cell wall from the medium. Exactly similar processes may occur during net inward transport of K^+ and Cl^-. The cause of the depolarization of the plasmalemma, when the inward transport of K^+ (linked to Cl^- influx) ceases in the dark might therefore be interpreted as an increase in effective K^+ activity just outside the plasmalemma. In SO_4^{2-} solutions, the depletion due to transport should also cease, accounting for the depolarization in SO_4^{2-}-FPW, which is somewhat greater than that in the dark. However, K^+ influx from SO_4^{2-}-FPW does not always decline, which is puzzling since the depolarized level of the plasmalemma, reached in SO_4^{2-}, is rather more positive than the calculated value of the Nernst potential for K^+. At this potential difference, and especially as the plasmalemma conductance always increased in SO_4^{2-}-FPW, an increased net efflux of K^+ would be expected. This has not yet been studied.

There are difficulties in interpreting the K^+ influx as being at all times under the control of the electrochemical potential gradient or permeability of the plasmalemma to K^+. The effects of N_2 bubbling on the K^+ influx are particularly difficult to interpret in this way. The plasmalemma became hyperpolarized without any increase in resistance, but nevertheless the K^+ influx decreased markedly. Furthermore, the decreases of K^+ and Cl^- influxes caused by the N_2 treatment were unequal (0.5 pmole·cm^{-2}·sec^{-1} and 1.0 pmole·cm^{-2}·sec^{-1} for Cl^- and K^+ respectively; the difference is statistically very significant). It is possible that lack of CO_2 causes a crossover to selective membrane permeability to some other ion, or a very strong "depleting" effect due to a sudden burst of inward transport, not detected in tracer experiments conducted 2–3 h after the start of N_2-bubbling.

TABLE VI

SENSITIVITY OF CELL FLUXES TO LIGHT/DARK, AND REMOVAL OF THE "COUNTERIONS"

State	*Ion*	*Influx light-sensitive*	*Influx sensitive to removal of "counterions"*	*Text reference*
A	Cl^-	+	+	Expt. 2
	K^+	+	+	
B	Cl^-	+	+	Expt. 3
	K^+	+	−	
C	Cl^-	−	−	Unpublished data
	K^+	+	−	
D	Cl^-	−	−	Expt. 1
	K^+	−	−	

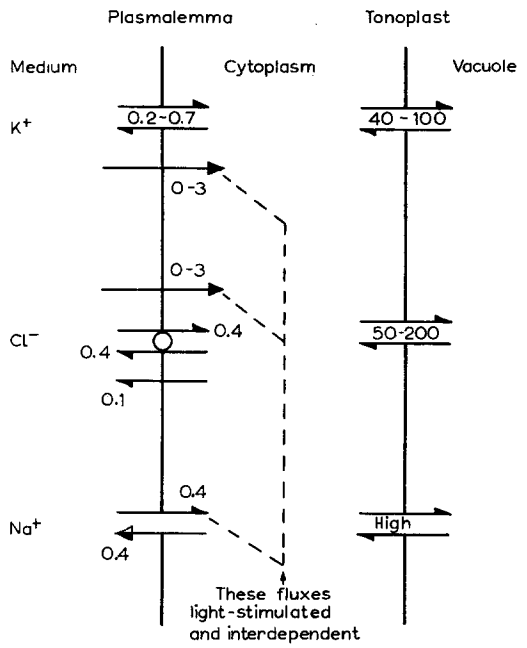

Fig. 5. A diagram summarizing the mean fluxes (pmole·cm^{-2}·sec^{-1}) of K^+, Cl^- and Na^+ which flow in and out of cells in FPW, in the light, for cells in State A (see text). The tonoplast fluxes are those found in the present work or (Cl^-) from a previous report[7]. In the dark the coupled influxes (dotted lines) reduced to zero, the passive efflux of Cl^- of 0.1 increased to about 0.4, while the exchange flux of Cl^- stayed at 0.4.

Na^+ extrusion

Because ouabain does not affect Na^+ efflux and K^+ influx, we see no reason to regard these two fluxes as being linked. The mechanism of the Na^+ extrusion pump in Chara cells differs radically in this respect from that in Nitella and Hydrodictyon.

Conclusions

Table VI summarizes the different interactions between K^+ and Cl^- influxes in Chara cells. In most experiments the cells were of the type described as 'State A', and Fig. 5 shows typical values of ion fluxes obtained with these cells. These values represent the situation where there is net influx of salt in the light. In States B–D of Table VI the K^+ and Cl^- fluxes are apparently independent. When one of these influxes changes without an equal change in the other there must be changes in other ion fluxes (*e.g.* K^+ efflux, Cl^- efflux) possibly with changes in the electrical properties of the cell membranes. The reason for the occurrence of several 'states' of Chara cells is obscure, but must be explained before any comprehensive theory of ion transport emerges. Electrical measurements revealed little difference between cells of States A and D, for example, but such measurements would not have detected increased turnaround in an exchange-diffusion system, which has been shown to account for some of the Cl^- influx.

Preliminary observations have suggested that cells in State D after harvesting (Cl^- influx high in the dark and not light-sensitive) may change to State A on storage in the laboratory in FPW.

ACKNOWLEDGEMENTS

This work is part of a joint project between the Schools of Biological Sciences at the University of Sydney, New South Wales, and at Flinders University, South Australia.

We are grateful for support from the Australian Research Grants Committee and the Nuffield Foundation.

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