THE EFFLUX OF CHLORIDE FROM CELLS OF *NITELLA* AND *CHARA*

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Summary

The efflux of chloride across the plasmalemma of internodal cells of *Nitella translucens* and of *Chara australis* has been measured under various conditions. It is large in the dark, and is reduced by light and by removal of the chloride ions from the external solution. Typical values for a chloride concentration of 1·3 mM in the external solution are: light, 0·6 pmole cm^{-2} sec^{-1}, rising in the dark to about 4·0 pmole cm^{-2} sec^{-1} for several hours, and finally steady in the dark at about 2·5 pmole cm^{-2} sec^{-1}. It is concluded that these changes are produced by changes in the permeability of the membrane to chloride ions. The two species give very similar results.

I. Introduction

In the extensive studies of ionic fluxes across the cell membranes of *Nitella translucens* (MacRobbie 1962, 1964, 1965; Spanswick and Williams 1964), little attention has been paid to the chloride efflux. A net outward movement of ions from *Nitella* cells in the dark has been shown by Tazawa and Nagai (1960), Tazawa (1961), and Nagai and Tazawa (1962), and these workers inferred that the anion released was chloride; but their method did not give the individual fluxes.

The interpretation of membrane potential measurements in *Chara* and *Nitella* internodes is often difficult, especially under conditions different from those used by Hope and Walker (1961). It seems clear that for the interpretation of membrane potentials we need information on the fluxes of chloride, since these are comparable in magnitude with the fluxes of sodium and potassium. Briggs (1962) has drawn attention to the role which the inward chloride pump would play in determining the membrane potential, if this pump were electrogenic. A brief experimental investigation into this question was begun, but it at once became obvious that the chloride efflux must also be studied, and the work reported here was begun.

In this paper it is assumed for the purposes of discussion that the chloride efflux is passive. This is very probable, since in these cells there is an outwardly directed gradient of electrochemical potential for chloride of very considerable magnitude. The potential difference across the plasmalemma is about −140 mV, inside negative (Spanswick and Williams 1964), and the concentration of chloride in the cytoplasm is *c.* 80 mM (with 1·3 mM outside). Thus the difference between the membrane potential and the equilibrium potential for chloride is about 245 mV. Further, as the present results clearly show, there is no positive coupling between the chloride efflux and the supply of light energy.

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II. Material and Methods

Plants of *Nitella translucens* Agardh were collected from a loch in Perthshire, Scotland, and were kept in the laboratory in dim light in an artificial pond water containing 1·0 mM NaCl, 0·1 mM KCl, and 0·1 mM $CaCl_2$. For these experiments long internodal cells were selected, and the adjacent cells removed.

Plants of *Chara australis* R.Br. var. *australis* were grown in the laboratory in cultures containing artificial pond water and river mud. They were used in many experiments when *Nitella* was not available. There seems to be no important difference between the two species as far as the chloride efflux is concerned.

The cells were loaded with ^{36}Cl by long soaking in a solution resembling artificial pond water, in which the chloride was labelled with ^{36}Cl. In early work the cells were soaked for 4 weeks in a solution containing 10 mM NaCl, 0·1 mM KCl, and 0·1 mM $CaCl_2$, with the chloride at a specific activity of about 0·5 mc/g. The solution was renewed weekly. For most experiments, however, the cells could be loaded in 1 week in artificial pond water, with a specific activity of 5·0 mc/g chloride.

Upon removal from the labelling solution the cells were inspected and then washed for 24 hr in inactive artificial pond water. Each cell was then placed in a groove in a wax block which held about 1 ml of solution. This solution was replaced hourly, and the solution withdrawn was dried on a planchet for counting. During the 16 hr "overnight" period each cell remained in 10 ml of solution, of which 1 ml was later counted.

Radioactivity was determined by counting with an end-window Geiger tube with low background shield. Counting was generally carried out to an accuracy of $\pm 5\%$, but a number of samples of low activity were counted to only about $\pm 15\%$. Most flux results were the mean of four or more successive hourly samples.

At the end of each experiment samples of vacuolar sap were taken from each cell, and the specific activity of the chloride was determined, the concentration being measured by electrometric titration. The specific activity of the vacuolar sap was used in the calculation of the absolute efflux, making the assumption that the specific activity of chloride in the cytoplasm was nearly equal to that of the chloride in the sap. MacRobbie's results suggest that this should be the case, with only a small percentage error.

Chloride activity measurements were carried out on samples of extracted sap and cytoplasm from a number of cells. The cells had 4 hr in light or dark, and were then gently centrifuged (about 5 min at about 50 *g*) to bring the flowing cytoplasm to one end. The cell was pinched and cut at the sap/cytoplasm boundary, and samples of sap and of cytoplasm were collected in a fine glass capillary (Microcap). The method followed Spanswick and Williams (1964). The chloride activity in the sample was then found by measuring the potential difference between a 1M KNO_3 bridge at one end of the sample, and a fine chlorided silver wire introduced into the other end. The electrode system was frequently calibrated with standard solutions.

The influx of chloride was measured by the method of MacRobbie (1965) in which the cells were soaked for 2–4 hr in the labelled solution, washed, and the whole cell then counted.

All flux measurements were carried out at 15°C. Cells which received "light" were exposed to about 100 f.c. of fluorescent light (National "white" tube).

The solutions were made up in doubly glass-distilled water with reagent grade salts. The benzene sulphonate salts were purified by recrystallization, and then gave colourless solutions.

Dilute alkali was added to all the samples before they were dried on planchets.

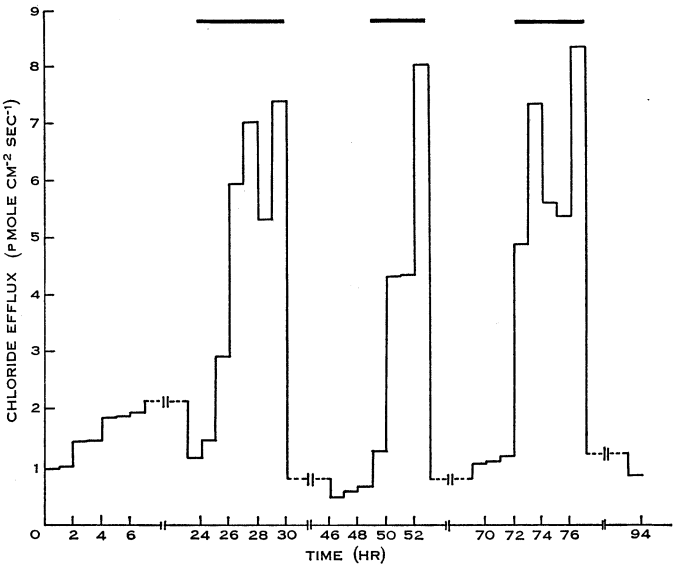

Fig. 1.—Efflux of chloride from cell of *Nitella translucens* as a function of time. Dark periods indicated by heavier horizontal rules. Cell in chloride artificial pond water. Dashed segments of histogram with break indicate average efflux during 16 hr overnight periods.

III. Results

The efflux of chloride from cells of *Nitella translucens* and *Chara australis* consistently follows the patterns illustrated in Figures 1–3. At the beginning of a light period the efflux falls, either rapidly or over 2–3 hr, to a low, more or less steady level, of the order of 0·5 pmole cm^{-2} sec^{-1}. At the beginning of a dark period it rises during 2–4 hr to a very high level and maintains a high but slightly reduced level for many hours. There is considerable variation in absolute efflux from cell to cell, but this pattern of behaviour is quite consistent, and is very similar in the two species studied. The mean effluxes during successive light, dark (2nd–5th and 5th–23rd hr inclusive), and light periods with cells bathed in chloride artificial pond water are given in Table 1.

It is necessary to consider whether the changes in radioactivity leaving the cell are changes in chloride efflux with a more or less constant specific activity, as has been assumed in the presentation so far, or whether the observed changes might

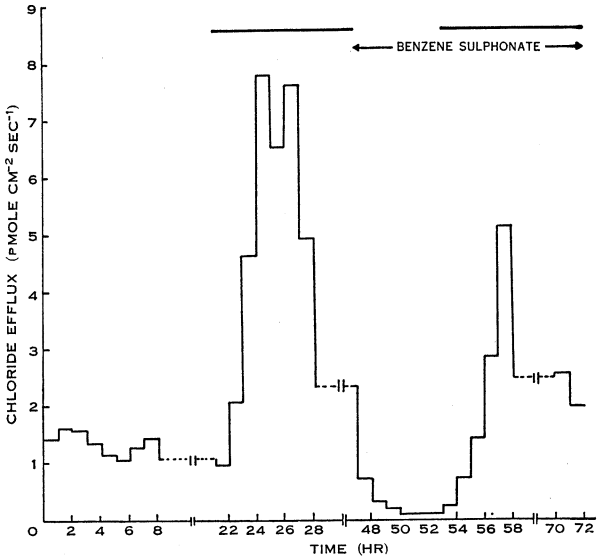

Fig. 2.—Efflux of chloride from cell of *Chara australis* as a function of time. Dark periods indicated by heavier horizontal rules. Cell in chloride artificial pond water except where chloride replaced by benzene sulphonate as indicated by arrows. Dashed and broken segments indicate 16 hr overnight periods.

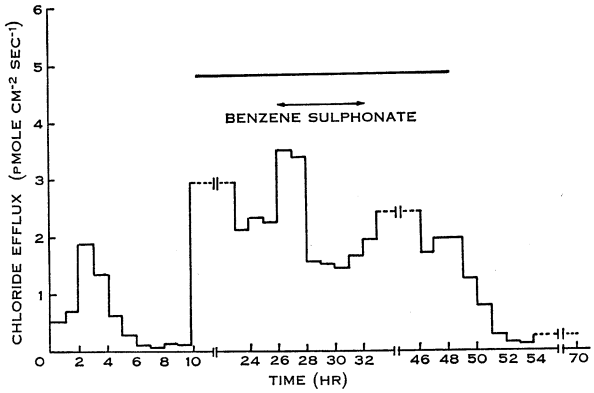

Fig. 3.—Efflux of chloride from cell of *Chara australis* as a function of time. Dark period indicated by heavy horizontal line. Other conditions as for Figure 2.

be changes in the specific activity of a constant chloride efflux. The latter might arise in two ways:

(1) the specific activity of the effluxing chloride might be equal to that of the sap chloride in the dark, and much lower in the light (perhaps because the labelled chloride just inside the plasmalemma is mixed, in the light, with the inactive chloride pumped in);

(2) the specific activity of the effluxing chloride might be equal to that of the sap chloride in the light, and much higher in the dark (perhaps because chloride of high specific activity is released into the cytoplasm in the dark, e.g. by the chloroplasts).

We can rule out (1) by considering the experiments illustrated in Figures 2 and 3. When the cell is bathed in a chloride-free artificial pond water containing sulphate or benzene sulphonate as anion the depression of the efflux by light persists. Mean values (±S.E.) for eight determinations of efflux under these conditions were found

TABLE 1

EFFECT OF LIGHT AND DARK ON CHLORIDE EFFLUX FROM *NITELLA* AND *CHARA* CELLS INTO CHLORIDE ARTIFICIAL POND WATER

Cell	No. of Determinations	Mean (±S.E.) Chloride Efflux (pmole $cm^{-2} sec^{-1}$)			Ratio of Dark Efflux to Light Efflux
		Light	Dark	Light	
Nitella	11	0·69±0·11	3·9±0·6 *	0·65±0·09	5·8±0·3
Chara	5	0·92±0·33	3·9±0·9 *	0·37±0·08‡	6·1±0·8
Nitella	2	0·35	0·65 †	0·2	2·4
Chara	7	0·81±0·21	2·5±0·4 †	0·45±0·11	4·3±0·7

* Mean of measurements during 2nd–5th hr of dark period.
† Mean of measurements during 5th–23rd hr of dark period.
‡ Mean for four determinations.

to be 1·7±0·4 pmole $cm^{-2} sec^{-1}$ in the dark, falling to 0·10±0·02 pmole $cm^{-2} sec^{-1}$ in the light (ratio of dark to light effluxes 19±4). In these solutions there is no question of dilution of the internal chloride. Case (2) is equally unlikely. MacRobbie has shown that the specific activity of chloride in the chloroplasts is equal to that in

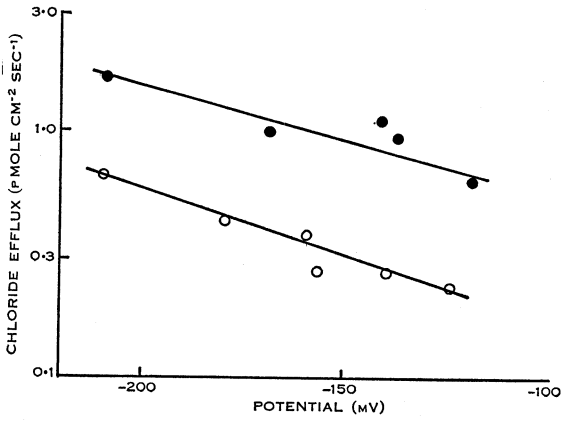

Fig. 4.—Efflux of chloride from cell of *Chara australis*, as a function of membrane potential. Cell in chloride artificial pond water. ○ Efflux in light. ● Efflux in dark after 8 hr darkness. Membrane potential controlled by voltage clamping.

the bulk cytoplasm, and (Fig. 3) the high efflux in the dark persists for so long that an impossibly large reservoir of high specific activity would have to be postulated.

The change in chloride efflux being accepted as real, it is of interest to examine any dependence of the driving force on light. The membrane p.d. is one component of the driving force, but it is most unlikely to change so much with light as to produce the observed flux changes. This is confirmed by Figure 4 (from work by Hope and

Walker, unpublished data). In this figure the efflux of chloride is shown as a function of membrane potential, in light and in dark, and it is seen that the efflux is relatively insensitive to changes in membrane potential, and that at a given membrane potential it is considerably smaller in the light.

The other component of the driving force on the efflux is the chloride activity in the cytoplasm. This was determined by the method described, on batches of *Nitella translucens* cells which had had 4 hr pretreatment in either light or dark. The method gives a sample of cytoplasm free of chloroplasts, although contamination by some sap is likely. It should, however, detect any large change in cytoplasmic chloride. The mean activities (±S.E.) for 16 light and 11 dark determinations are given in the following tabulation:

	Chloride Activity (mM)	
	Sap	Cytoplasm
Light	162±7	87± 7
Dark	180±9	91±11

It is clear that there is no significant difference between the chloride activities in the light and in the dark.

MacRobbie (1965) has shown that the light-promoted chloride influx is inhibited selectively by Diuron (dichlorophenyldimethylurea), and that the light-promoted potassium influx is inhibited selectively by imidazole. The effects of these inhibitors on the light-reduced chloride efflux were determined (Table 2) together with comparison experiments on the chloride influx (Table 2).

TABLE 2

EFFECT OF LIGHT AND INHIBITORS ON CHLORIDE FLUXES IN *NITELLA TRANSLUCENS*

Number of cells measured given in parentheses

Light Treatment	Inhibitor Used	Mean Influx (±S.E.) (pmole cm^{-2} sec^{-1})	Mean Efflux (±S.E.) (pmole cm^{-2} sec^{-1})
Light	No inhibitor	0·52±0·07 (5)	0·45±0·03 (18)
	Imidazole, 3×10^{-4}M	0·76±0·08 (6)	0·65±0·11 (4)
	Diuron, 5×10^{-7}M	0·12±0·03 (5)	1·16±0·13 (4)
	Diuron, 10^{-6}M	0·13±0·03 (5)	1·59±0·36 (4)
Dark	No inhibitor	0·09±0·02 (10)	1·73±0·21 (6)

IV. DISCUSSION

These experiments demonstrate the large effect of light on the efflux of chloride across the plasmalemma; the dark efflux is reduced by light by a factor of about 4. Further, the replacement of external chloride by sulphate or benzene sulphonate

reduces the efflux in the light by a factor of about 4; it appears to reduce the efflux in the dark by a factor of about 2, but this is not well established by the present results.

The effect of light here described is the inverse of the effect of light on the chloride influx, which is increased by light from the dark level of about 0·1 pmole cm^{-2} sec^{-1} to about 0·5 pmole cm^{-2} sec^{-1} or more. The concentrations of Diuron and imidazole used also have inverse effects, i.e. low concentrations of Diuron substantially inhibit the light effect on both influx and efflux, while imidazole does not inhibit either light effect. Thus, following MacRobbie's (1965) scheme, the light effect on the chloride efflux seems to depend on electron transport rather than on photophosphorylation.

It is clear that there is no direct causal relation between high influx and low efflux, however, since the removal of external chloride ions in the light abolishes the influx and reduces the efflux (Table 3). The results described here do not establish a

TABLE 3

EFFECT OF LIGHT AND IMPERMEANT ANIONS ON CHLORIDE EFFLUX FROM CELLS OF *CHARA AUSTRALIS*

Artificial Pond Water Anion	No. of Determinations	Mean Efflux (±S.E.) in Light (pmole cm^{-2} sec^{-1})	Ratio of Efflux in Sulphate or Benzene Sulphonate to Efflux in Chloride	No. of Determinations
Chloride	10	0·61±0·12	Sulphate, 0·16±0·04	3
			Benzene sulphonate, 0·20±0·04	7
Sulphate or benzene sulphonate	10	0·11±0·02	Mean ratio: 0·19±0·02	10

mechanism for the efflux light effect, but they contain strong evidence that it is not produced by a change in driving force, either membrane potential or internal chloride activity. Hence it is concluded that the action of light is to reduce the permeability of the membrane to chloride ions. This action of light appears to be related to electron transport, as is the energy supply for the chloride influx pump. It seems unlikely that the two effects of light are unrelated, and the possibility is suggested that the large dark efflux occurs via the carrier mechanism of the influx pump when its energy supply is removed.

The effect of sulphate suggests a phenomenon like exchange diffusion; but in the light, exchange diffusion fluxes of about 0·4 pmole cm^{-2} sec^{-1} would be required, while the maximum exchange diffusion fluxes in the dark would be about 0·1 pmole cm^{-2} sec^{-1}. So it would be necessary to postulate a light-dependent exchange diffusion. Alternatively the removal of external chloride ions may reduce the chloride permeability of the membrane, as appears to occur in frog muscle (Spurway 1965).

The change in efflux with light is of a sufficient magnitude to affect the membrane potential of the cell. For a change in efflux of 3·0 pmole cm^{-2} sec^{-1} is equivalent to a change in membrane current of 0·3 μA cm^{-2}, and in a cell whose membrane resistance is 50 kΩ cm^2 this will produce a potential change of 15 mV.

A cell in which no other changes occurred would thus be expected to depolarize in the dark by some 10–20 mV through chloride efflux changes alone. Such changes in membrane potential have been observed in *Nitella flexilis* (Nagai and Tazawa 1962) and are sometimes seen in *N. translucens* (Walker, unpublished data).

V. Acknowledgments

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