IONIC RELATIONS OF CELLS OF *CHARA AUSTRALIS*

X. EFFECTS OF BICARBONATE IONS ON ELECTRICAL PROPERTIES

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Summary

When bicarbonate ions were added to the external medium the plasmalemma of cells of *Chara australis* became hyperpolarized. The potential difference (p.d.) frequently changed from −150 or −160 mV to −200 or −220 mV. An increase in membrane resistance also occurred.

In the hyperpolarized state the plasmalemma was almost unresponsive to changes in potassium concentration in the medium.

Dark conditions slowed or inhibited the change in p.d. due to bicarbonate. A large, reversible increase in the resistance of the plasmalemma accompanied a transition from light to dark when the membrane was hyperpolarized.

Hyperpolarization was partly abolished reversibly by subsequent addition of appropriate concentrations of *o*-phenanthroline, monuron (1-*p*-chlorophenyl-3,3-dimethylurea), or hydroxylamine.

It is postulated that an active "electrogenic" pump accumulates bicarbonate ions, which are accompanied by a passive flux of potassium and sodium ions. The latter flux constitutes an electric current which hyperpolarizes the plasmalemma. The active pump is dependent on energy from photosynthesis.

I. INTRODUCTION

In a previous paper (Hope and Walker 1961) it was shown that under certain conditions the electric potential difference between the vacuole and external medium in the giant coenocytes of *Chara australis* follows changes in the ratio of potassium to sodium in the external medium. The potential changes and accompanying changes in the electric resistance were shown to be approximately those expected from a membrane model with a linear potential gradient and with passive permeability to potassium and sodium ions. Other investigations have shown that most of the potential difference (p.d.) and change in p.d. is across the plasmalemma. The same applies to the electric resistance—that of the tonoplast being generally much less than that of the plasmalemma (Walker 1955, 1960; Findlay and Hope 1964).

Under some conditions the simple model fails to describe the observations. Hope and Walker (1961) stated that removal of calcium ions from outside the cell, probably including those adsorbed by the cell wall, was necessary before the plas-

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malemma became responsive to potassium. Such absence is not always essential, but calcium ions frequently have the effect of reducing the p.d. change in *Chara* cells for a given change in K^+/Na^+ (compare Kishimoto 1959), probably through an alteration in permeability to potassium (Hope 1963).

The present paper describes further circumstances in which the electrical properties of the plasmalemma are not matched by the simple model; an explanation in terms of two alternative mechanisms is attempted and the effects of bicarbonate ions are discussed in relation to those of light and dark on the p.d. in *Nitella*, described by Nagai and Tazawa (1962).

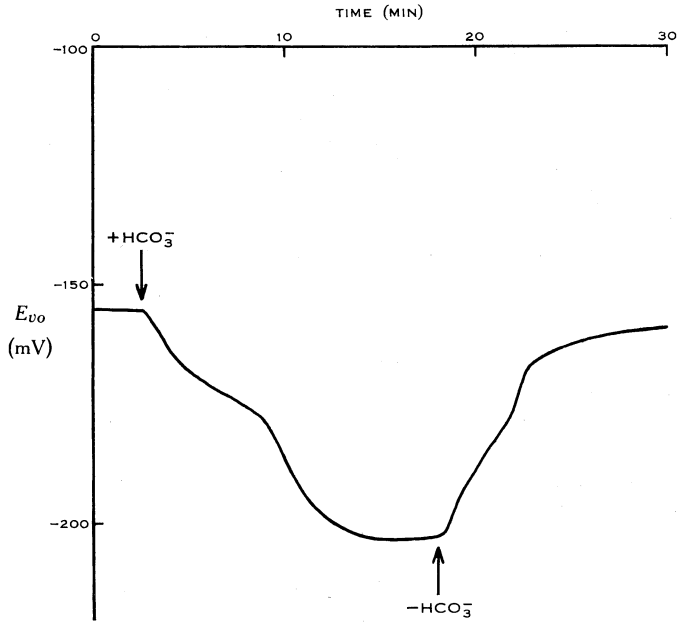

Fig. 1.—Vacuolar potential difference plotted against time showing the effects of adding and removing bicarbonate ions. At the first arrow a change was made from 1·1 mN Cl^- to 0·6 mN Cl^-, 0·5 mN HCO_3^-, K^+ and Na^+ being 0·1 and 1·0 mN throughout. At the second arrow the chloride was restored and bicarbonate removed.

Blinks (1940), in a review of the relation between metabolism and bioelectric phenomena, referred to observations that in *Valonia*, CO_2 and weak acids may reverse the normal p.d. and cause the vacuole to become negative; in *Nitella* there were also effects of CO_2 and of strong light on the resistance. They were suspected to be caused by changes in internal pH.

II. Material and Methods

These have been described in earlier papers (see Findlay and Hope 1964). Artificial pond waters containing bicarbonate ions were made up freshly, as stale solutions led to variable results in early experiments. The pH of fresh bicarbonate

solutions was for 0·1 mN, 7·1–7·2; for 0·5 mN, 8·6. In a few experiments 0·1 mN K_2CO_3 was used, the initial pH being about 9·5. The pH of all-chloride media was 5·8–6·0.

The p.d.'s quoted have the sign of the inside of the cell relative to the outside.

III. Results

(a) *Potential Difference and Resistance in Solutions containing Bicarbonate Ions*

Figure 1 shows the vacuolar p.d. in a typical experiment, plotted against time. When a change was made from an all-chloride medium to one in which HCO_3^- was substituted for some chloride, a large hyperpolarization occurred. This effect was obtained in more than 50 cells with bicarbonate concentrations ranging from 0·05 to 1·0 mN. The presence of calcium, strontium, or magnesium, or the absence of all,

Table 1

Comparisons of vacuolar potential difference (E_{vo}) and resistance (r_o) of chara cells in media containing either all chloride or chloride plus some bicarbonate ions

Date of Experiment	Concentration of Ions in Medium (mN)		E_{vo} (mV)	r_o (kΩ.cm²)
	Chloride	Bicarbonate		
29.ix.61	1·1	—	−168	12
	0·6	0·5	−215	25
5.x.61	1·1	—	−157	6·3
	0·6	0·5	−200	12·5
17.x.61	1·6	—	−161	6·9
	1·1	0·5	−192	8·6
26.ii.62	1·1	—	−154	18
	0·6	0·5	−205	24
1.iii.62	1·1	—	−160	3·4
	1·0	0·1	−220	5·5
14.iii.62	1·1	—	−151	4·3
	1·0	0·1	−209	6·5
15.iii.62	1·1	—	−160	4·6
	1·0	0·1	−210	5·6

did not influence the change in p.d., which was commonly from −150 or −160 mV to −200 or −230 mV. For the above experiments the cells were in light, which was usually microscope illumination plus room lighting. The intensity was not measured. The hyperpolarization was reversed on removing the HCO_3^-, as seen in Figure 1. Most experiments were made using 0·1 mN HCO_3^- since this caused only a small change in the chloride concentration externally.

The cell resistance (that of the tonoplast and plasmalemma in series) and vacuolar p.d. are compared in Table 1 for cells placed first in all-chloride medium and then in a medium with bicarbonate substituted for some chloride ions. As well as the pronounced hyperpolarization caused by bicarbonate, the resistance was also increased, but not so markedly by 0·1 as by 0·5 mN HCO_3^-.

(b) Effect of Changes in Potassium Concentration

Figure 2 shows the vacuolar p.d. plotted against time in a representative experiment. In the presence of 0·5 mN HCO_3^-, the change from 0·1 mN K^+–1·0 mN Na^+ to 1·0 mN K^+–0·1 mN Na^+ caused little change in p.d., with (Fig. 2) or without calcium (see below).

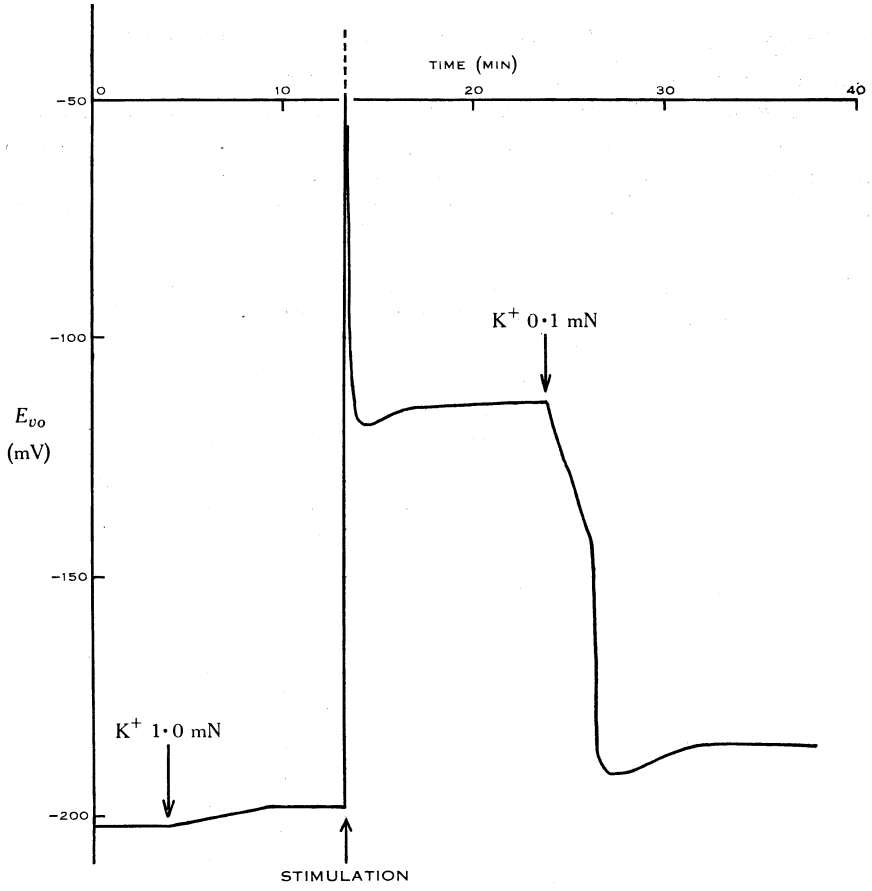

Fig. 2.—Potential difference plotted against time, showing the response to change in the K^+/Na^+ ratio, before and after an action potential, with calcium present.

Cells hyperpolarized in this way, and with the high concentration of K^+ in the medium, were stimulated to produce an action potential. This was done by the passage of a short pulse of current tending to send positive ions out of the cell. In the presence of external calcium ions, at 0·5–1·0 mN, two responses have been observed: either the p.d. returned to the hyperpolarized level after the action potential or, more often (e.g. Fig. 2) stayed depolarized at −100 to −120 mV, a level characteristic of the potassium-responsive membrane when in 1·0 mN KCl–0·1 mN NaCl (Hope and Walker 1961).

At this depolarized level, on substituting 0·1 mN K^+–1·0 mN Na^+ for 1·0 mN K^+–0·1 mN Na^+, the cell p.d. always responded as shown in Figure 2. Initially the change appeared to be approximately exponential, as if tending towards the usual level of −150 to −170 mV [cf. Fig. 1(*b*) of Hope and Walker 1961], but a "slip" occurred later during which the p.d. changed from about −180 to −220 mV in about 20 sec. When HCO_3^- was present at 0·1 mN this part of the p.d. change was slower. The total change in p.d. consequent on changing the potassium concentration from 1·0 to 0·1 mN under these conditions was often as much as −110 mV.

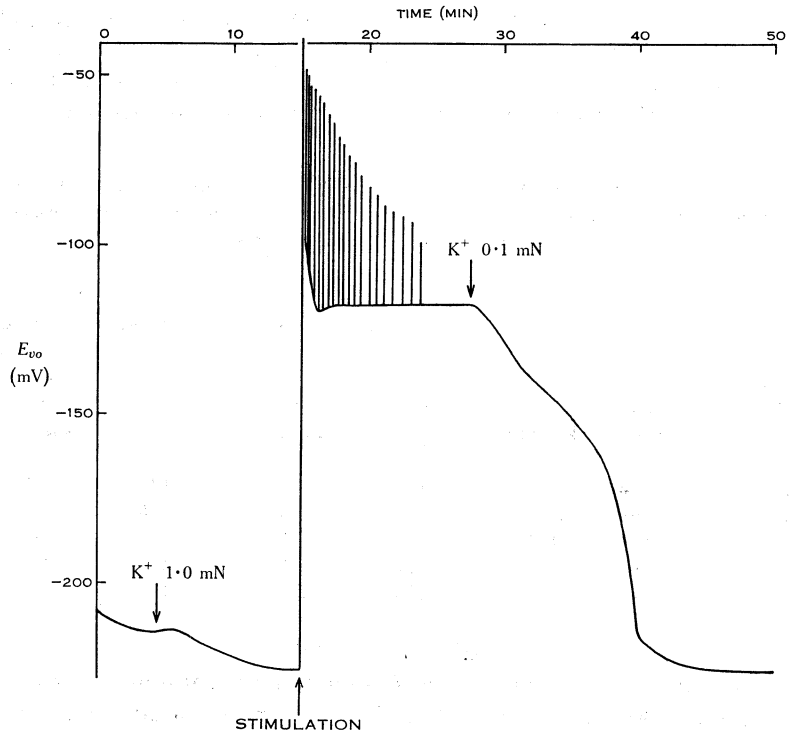

Fig. 3.—Potential difference in 0·5 mN HCO_3^-, without calcium, illustrating the effect of change in the K^+/Na^+ ratio, before and after stimulation.

In a series of solutions identical with those above except that calcium was absent, the response of the cell p.d. to an increase in potassium concentration was small. Initiation of an action potential evoked a train of spontaneous action potentials (Fig. 3) of decreasing amplitude until finally the magnitude of the action potential was zero. The p.d. then remained at the depolarized level of −100 to −120 mV. On restoration of 0·1 mN K^+–1·0 mN Na^+ the p.d. returned to the hyperpolarized level, similar to that observed when calcium was present. The changes in resistance and p.d. between the hyperpolarized state (low and high potassium) and the depolarized state are summarized in Table 2. The mean change in E_{vo} between low and high potassium with bicarbonate present was, in these five experiments, +2 mV, with a standard error of ±4 mV; in the absence of bicarbonate the value is from +40 to +50 mV (Hope and Walker 1961).

(c) *Effect of Light and Dark in Presence of Bicarbonate*

Dark conditions generally slowed down or inhibited the hyperpolarization due to HCO_3^- (Fig. 4). When the HCO_3^- concentration was 0·1 mN, illumination caused an immediate drift in p.d., which "slipped" to −215 mV. In the dark, in the presence of bicarbonate, cells were potassium-responsive without electrical stimulation. However, in 0·1 mN K^+–1·0 mN Na^+ and in the hyperpolarized condition, a change from light to dark did not cause a large change in p.d. Sometimes a drift from about −220 to −200 mV was observed. Large changes in cell resistance were associated with light–dark or dark–light transitions, an observation made also by Walker (unpublished data, 1960). The changes in resistance occurred with or without calcium ions in the medium. Figure 5 illustrates this effect. The effect of light and dark on the p.d. and resistance was almost negligible in media not containing bicarbonate ions.

TABLE 2

CHANGES IN POTENTIAL DIFFERENCE AND RESISTANCE OF CHARA CELLS IN MEDIA CONTAINING BICARBONATE IONS AND EITHER LOW OR HIGH CONCENTRATIONS OF POTASSIUM IONS (K_o), PRIOR TO AND FOLLOWING STIMULATION

Date of Experiment	K_o = 0·1 mN		K_o = 1·0 mN (before action potential)		K_o = 1·0 mN (after action potential)	
	E_{vo} (mV)	r_o (kΩ.cm²)	E_{vo} (mV)	r_o (kΩ.cm²)	E_{vo} (mV)	r_o (kΩ.cm²)
17.vii.61	−193	12·0	−198	13·6	−130	0·7
24.viii.61	−199	22·7	−184	19·3	−115	3·3
1.x.61	−210	16·0	−204	18·7	−112	2·3
17.x.61	−198	7·3	−207	7·8	−135	2·8
27.ii.62	−217	13·4	−216	11·9	−118	2·4

(d) *Effect of Inhibitors*

The effect of several substances which inhibit photosynthesis was investigated. Figure 6 shows the response of cell p.d. to the addition of 0·3 mM *o*-phenanthroline [which inhibits oxygen evolution (Warburg and Lüttgens 1946)] to a medium comprising 0·1 mN $KHCO_3$ and 1·0 mN NaCl. A summary of the effect of this and other substances is given in Table 3. In all instances the effect of *o*-phenanthroline up to 0·5 mM was to increase the p.d. from the hyperpolarized level, by amounts depending on its concentration, towards −150 or −140 mV. The change was reversed on withdrawing the inhibitor. The concentration of 0·5 mM appeared to be critical, since greater amounts of *o*-phenanthroline had the effect of rapidly depolarizing the membrane to values of the order of −70 mV; the effect was only slowly reversible, or irreversible in periods of 30–60 min (the first experiment quoted in Table 3 is an exception). *o*-Phenanthroline (up to 0·4 mM) added to 0·1 mN KCl – 1·0 mN NaCl medium, with or without 0·5 mN $CaCl_2$ did not depolarize the membrane. A small hyperpolarization was usually noted. If HCO_3^- were now added, further hyperpolarization did not occur. Cell membranes in which the hyperpolarization had been reduced or abolished by *o*-phenanthroline were responsive to changes in the K^+/Na^+ ratio, without the need of stimulation.

(*e*) *Location of the Hyperpolarizing Membrane*

Following the development of a technique for making simultaneous measurements of the p.d.'s across each of the plasmalemma and tonoplast (Findlay and Hope 1964) it has been demonstrated that during the hyperpolarization by bicarbonate solutions it is the p.d. across the plasmalemma which is affected, that across the tonoplast being small and constant at 0–10 mV. All the measurements in Sections III(*a*)–III(*d*) are of the p.d. between the vacuole and medium, but may be taken as closely approximating that across the plasmalemma. Low values of resistance such as in Table 2 may be considerable overestimates of the plasmalemma resistance, due to an unknown contribution from the tonoplast. In the experiments of Findlay and Hope (1964), the tonoplast resistance was usually not more than 1 $k\Omega.cm^2$ in media containing 0·1 mN K^+.

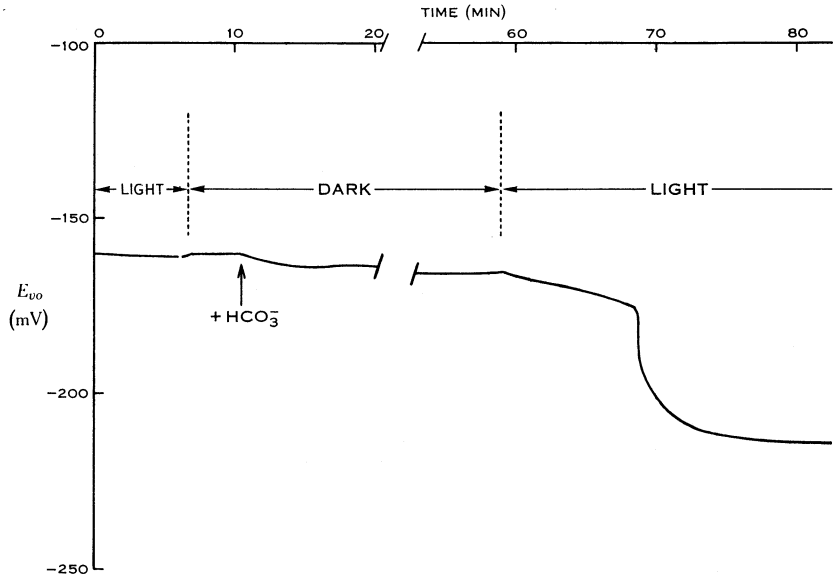

Fig. 4.—Effect of added HCO_3^- on the potential difference when the cell is in the dark.

IV. Discussion

It has been shown that when small amounts of bicarbonate ions are substituted for some of the chloride in KCl–NaCl media, the p.d. across the plasmalemma increases, the cytoplasm becoming more negative with respect to the medium. The change is from —150 or —160 mV to —200 or —230 mV. At the former levels, in the all-chloride media, the plasmalemma is responsive to changes in K^+/Na^+ concentration ratio (Hope and Walker 1961; Findlay and Hope 1964) and the p.d. is given approximately by:

$$E_{co} = 58 \log_{10}(K_o + \alpha Na_o)/(K_c + \alpha Na_c), \quad (1)$$

where *o* refers to outside and *c* to cytoplasmic ionic activities, and α is the permeability ratio P_{Na}/P_K. Values for α and $K_c + \alpha Na_c$ have been estimated to be about

0·1 and 100–120 mN respectively, agreeing fairly well with flux measurements or direct observation of ion activities in similar cells (MacRobbie and Dainty 1958; MacRobbie 1962; Hope 1963).

For an analogous equation to hold for the hyperpolarized membrane, an enormous value of $K_c+\alpha Na_c$ follows from equation (1) since the plasmalemma is now insensitive to the K^+/Na^+ ratio and α must be nearly unity. If α were unity and $E_{co} = -220$ mV, K_c+Na_c would have to be of the order of 10N, which is impossible.

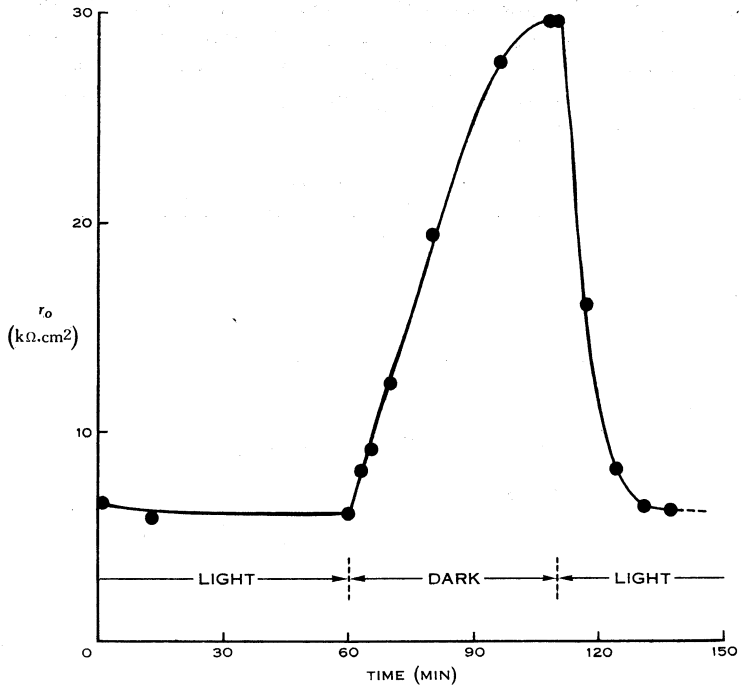

Fig. 5.—Effect of light to dark and dark to light transitions on the cell resistance, when bicarbonate ions (0·1 mN) are present.

It may be concluded that P_K and P_{Na} are small compared with the permeability to some other ion, or alternatively, that the p.d. is largely determined by an active transport system causing an influx of chloride or bicarbonate, or an efflux of sodium (cf. Briggs 1962). The proposals will be considered in turn.

(a) *Passive Fluxes of some Other Ion Determining the Potential Difference*

The plasmalemma has been shown to be unresponsive to changes in the K^+/Na^+ ratio when hyperpolarized. The p.d. was also unchanged by Cl^-/Br^- substitutions or $Ca^{2+}/Mg^{2+}/Sr^{2+}$ substitutions. It could therefore be proposed that the plasmalemma becomes specifically permeable to hydrogen ions (either in the hydrated form or as protons) and that the membrane p.d. is given by:

$$E_{co} = 58 \log_{10} (H_o/H_c) \quad (2)$$

$$= 58 (pH_c - pH_o). \quad (3)$$

A value for E_{co} of −220 mV then corresponds to a pH_c of 3·2 when the pH of the medium is 7, which seems unlikely but not impossible.

There is little evidence in the literature bearing on proton conduction through cell membranes. Eigen and De Maeyer (1956) have shown that the mobility of protons in ice is high compared with that in water and would thus be expected to be high in a system where water molecules were ordered, with frequent hydrogen bonds.

Such a system, if present in membranes (or in pores in membranes), would not be expected to provide a conductivity as high as observed, even with 10^{-3}N acid on the inside of the membrane.

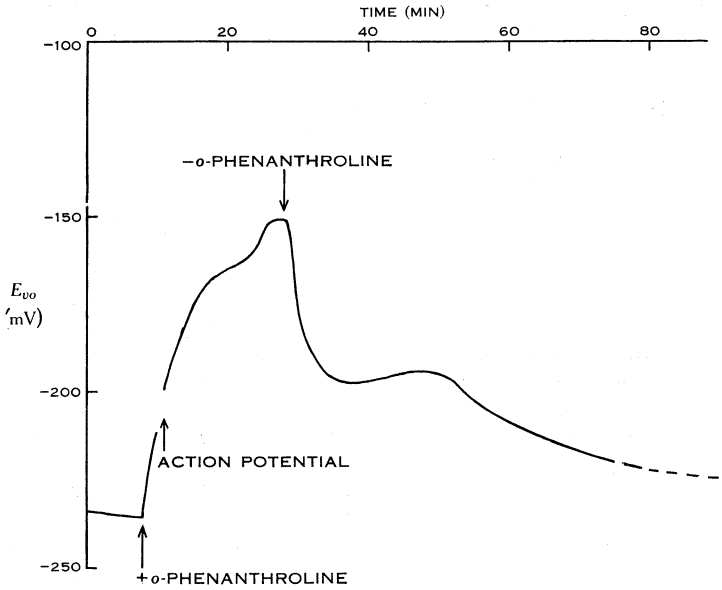

Fig. 6.—Effect on the potential difference of 0·3 mM *o*-phenanthroline after hyperpolarization by HCO_3^-.

(*b*) *Potential Difference Determined by an Active Flux*

One of the schemes for accumulation of salts involves active transport of the anions with accompanying passive fluxes of cations (see Briggs, Hope, and Robertson 1961). Briggs (1962) has shown that such operation of an anion pump could alter the resting potential from that given by equation (1) to a value given by:

$$K_o+\alpha Na_o = -(RT/EF)(\Phi/P_K)[1-\exp(EF/RT)]+(K_c+\alpha Na_c)\exp(EF/RT), \quad (4)$$

where Φ is the active transport flux and the other symbols have the same significance as before; note that E has the opposite sign from that in the treatment by Briggs (1962), since the present E is the potential of the inside of the cell relative to the ouside. Let it now be supposed that the presence of bicarbonate ions results in a net influx of these or an increase in an active chloride influx. Let the steady active influx in light and in the presence of bicarbonate be Φ. (A passive flux of chloride or bicarbonate is not considered at present.) Using plausible values for the other parameters, such as $\alpha = 0{\cdot}1$, $K_c+\alpha Na_c = 120$, then as Φ/P_K increases from zero to $1{\cdot}5\times10^{-6}$ equiv. cm^{-3}, E_{co} goes from −161 to −215 mV; i.e. hyperpolarization occurs.

If P_K is of the order of 10^{-5} cm. sec^{-1}, as calculated from electrical measurements (Hope and Walker 1961) then Φ would have to be 15 pequiv. cm^{-2} sec^{-1}, a rather large value. If P_K is *c.* 10^{-6} then Φ need be only 1·5 units. Both these estimates of P_K are based on arbitrary assumptions but for present purposes it is not necessary to know the absolute value. There is the further difficulty that, in the hyperpolarized state, the plasmalemma is almost unresponsive to changes in the ratio of K^+/Na^+ concentration, whereas the p.d. calculated from equation (4) would decrease as K_o increased. One reason for this could be that P_K is decreased during the hyperpolarization. The potassium insensitivity and the observation that a sufficient change in E by means of a current pulse will depolarize the cell to a level where it is more permeable to

TABLE 3

EFFECT OF INHIBITORS OF PHOTOSYNTHESIS ON VACUOLAR POTENTIAL DIFFERENCE OF CHARA CELLS EXPOSED TO LIGHT

Media contained 0·1mN $KHCO_3$ and 1·0mN NaCl

Date of Experiment	Inhibitor	Concn. (mM)	E_{vo} (mV)			
			Before Inhibitor	During Inhibitor	After Inhibitor	All Chloride, No Inhibitor
14.iii.62	*o*-Phenanthroline	1·0	−210	−167	−204	−150
15.iii.62	*o*-Phenanthroline	0·3	−205	−160	−200	−148
19.iii.62	*o*-Phenanthroline	0·3	−230	−192	−210	−159
19.iii.62	*α,α*-Dipyridyl	1·0	−210	−179	−215	—
3.i.63	*o*-Phenanthroline	0·3	−236	−151	−226	−157
17.vi.63	Monuron	0·01	−236	−187	−224	−155
25.vi.63	*o*-Phenanthroline	0·4	−210	−154	−190	−150
25.vi.63	Monuron	0·01	−215	−197	−210	−155

potassium (Figs. 2 and 3) suggests that equation (4) should be modified by making P_K a function of E. Thereby the difficulty of insensitivity to changes in K^+/Na^+ ratio is overcome, the same hyperpolarization is obtained with smaller active fluxes, and most important, the "slippage" phenomenon (Fig. 2, etc.) is explained.

Briefly, use of equation (4), with P_K now decreasing as E becomes more negative, shows that as Φ rises from zero to its final value, E drifts from −161 mV to a more negative value with a point of inflexion, the final value of E being dependent on the magnitude of Φ and P_K, P_{Na}. To give an example of how this can occur, P_K may be taken as an arbitrary function of E:

$$P_{K(E)} = P_{K(180)} \exp[(E+180)/12], \tag{5}$$

where $P_{K(E)}$ is the permeability at potentials E, and $P_{K(180)}$ that at −180 mV or more positive. Equation (5) makes $P_{K(220)}$ about one-tenth of $P_{K(180)}$ and hence $\alpha = 1$, as required. To predict a time course for potential change, it is reasonable to take Φ arbitrarily as a negative exponential function of time:

$$\Phi_t = \Phi[1-\exp(-kt)], \tag{6}$$

where k is a constant. Then E_{co} is the function of time plotted in Figure 7, with $\Phi = 8$, 9, and $9{\cdot}5\times10^{-12}$ equiv.cm^{-2}.sec for curves A, B, and C, $P_{K(180)} = 10^{-5}$ and $P_{Na} = 10^{-6}$ cm. sec^{-1} = constant. It can be seen that above a certain value of Φ the p.d. slips, but below this a negative exponential decrease is observed. Steeper rates of potential change during the slip can be obtained by increasing the exponent in equation (5). While it is not claimed that P_K and Φ are the functions of potential and time of equations (5) and (6), it is apparent that a qualitative agreement with observation can be arranged.

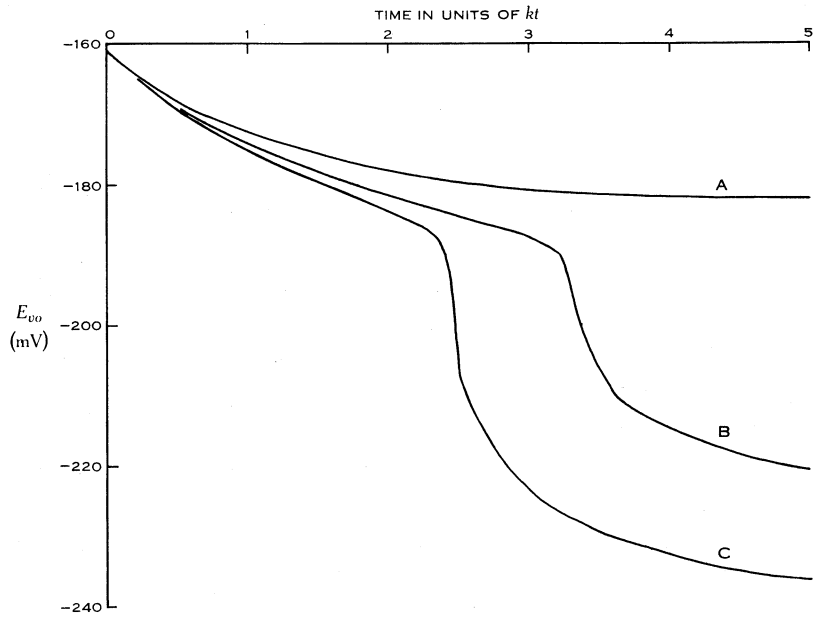

Fig. 7.—Theoretical curves relating the potential difference and time after adding bicarbonate ions, calculated from equations (4), (5), and (6). A, B, and C are curves showing the effects of increasing active influx.

Some preliminary observations have been made bearing on the fixation of HCO_3^- and on production of organic acids (Graham and Hope, unpublished data), to see what total amounts of HCO_3^- were taken up. When [^{14}C]HCO_3^- was placed in the external medium, *Chara* cells incorporated the label into various compounds in a 30-min period. The amount taken up corresponded to a flux of $1\text{–}10\times10^{-12}$ moles. cm^{-2}. sec^{-1}. The flux may have been of bicarbonate *ions* or of CO_2, depending on the pH near the chloroplasts. The uptake of radioactivity was reduced by *o*-phenanthroline or monuron (1-*p*-chlorophenyl-3,3-dimethylurea). Of the organic acids labelled, a small amount only of glycollate appeared in the external medium during the labelling period or in a subsequent 60-min period in unlabelled medium. The radioactivity in the released glycollate, however, was a minute fraction of the fixed carbon. Tolbert and Zill (1956) found glycollic acid to be released by *Chlorella* cells under somewhat similar conditions.

Thus the active flux necessary in equation (4) to hyperpolarize the membrane is within the limits of the observed uptake rates of bicarbonate. The effects of inhibitors are then apparently on the active flux, perhaps via the "free energy producing" reactions in photosynthesis. When Φ is reduced by this means the p.d. returns towards its usual resting level, in accordance with equation (4).

The effect of dark conditions in increasing membrane resistance on the presence of bicarbonate may be a direct effect on membrane permeability to K^+ and Na^+. In the dark a much smaller value of Φ suffices to cause hyperpolarization. It would be possible for the effects of dark on these two factors, P_K and Φ to cancel out (see equation (4) where Φ/P_K appears) and leave the p.d. almost unchanged, as observed.

Nagai and Tazawa (1962) have described effects of light and dark on the vacuolar p.d. and net influx of chloride in single *Nitella* cells. The media employed did not contain bicarbonate ions so the operation of an electrogenic chloride (influx) pump may be necessary to explain their observations. The active transport of chloride inwards is well substantiated in cells of the Characeae (MacRobbie and Dainty 1958; MacRobbie 1962, 1964) but it is not known whether this pump is electrogenic. In the *Chara* cells used here there was no effect of light or dark on the p.d. or resistance in all-chloride media; furthermore the removal of all external chloride and its replacement by bromide or other anions did not cause a detectable change in p.d., whereas the chloride influx in similar cells of *Chara* could be shown to change greatly during corresponding treatments (Hope, unpublished data). These data suggest that the pump transporting chloride inwards in *Chara australis* is either not electrogenic or else the resting passive fluxes are very high in relation to the extra passive flux occasioned by the pump.

Algal cells were reported by Steeman Nielsen (1947) to utilize bicarbonate ions directly in exchange for hydroxyl ions, the medium becoming more alkaline until pH 11 is reached. At this stage any bicarbonate remaining in the medium is converted to carbonate, which is not absorbed. The relation between this effect and the postulated electrogenic bicarbonate pump in *Chara* is not clear.

In summary, the following conclusions are reached if the mechanism discussed above in Section IV(*b*) is accepted:

(1) In the presence of dilute bicarbonate, an active transport system acts to pump bicarbonate ions or chloride ions into the cell.

(2) A net passive influx of potassium and sodium ions accompanies the active influx of anions.

(3) The active pump is "electrogenic" in that it causes the normal resting potential across the plasmalemma to increase markedly in magnitude.

(4) During the process of hyperpolarization caused by the operation of the active pump the passive permeability of the plasmalemma to potassium decreases; that to sodium may increase, since the resistance is seldom more than doubled during the hyperpolarization (Table 1); the decrease in P_K causes an acceleration or slip towards the final level of potential.

(5) The active transport is intimately linked to photosynthetic reactions, since both the electrical effects and the influx are reduced by *o*-phenanthroline, monuron, or hydroxylamine.

V. Acknowledgments

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VI. References

Blinks, L. R. (1940).—*Cold Spring Harb. Symp. Quant. Biol.* **8**: 204.
Briggs, G. E. (1962).—*Proc. R. Soc.* B **156**: 573.
Briggs, G. E., Hope, A. B., and Robertson, R. N. (1961).—"Electrolytes and Plant Cells." (Blackwell Scientific Publications: Oxford.)
Eigen, M., and De Maeyer, L. (1956).—*Z. Elektrochem.* **60**: 1037.
Findlay, G. P., and Hope, A. B. (1964).—*Aust. J. Biol. Sci.* **17**: 62.
Hope, A. B. (1963).—*Aust. J. Biol. Sci.* **16**: 429.
Hope, A. B., and Walker, N. A. (1961).—*Aust. J. Biol. Sci.* **14**: 26.
Kishimoto, V. (1959).—Ann. Rep. Sci. Works Fac. Sci. Osaka Univ. No. 7. p. 115.
Macrobbie, E. A. C. (1962).—*J. Gen. Physiol.* **45**: 861.
Macrobbie, E. A. C. (1964).—*J. Gen. Physiol.* **47**: 859.
Macrobbie, E. A. C., and Dainty, J. (1958).—*J. Gen. Physiol.* **42**: 335.
Nagai, R., and Tazawa, M. (1962).—*Plant & Cell Physiol.* **3**: 323.
Steeman Nielsen, E. (1947).—*Dansk. Bot. Arkiv.* **12**: 5.
Tolbert, N. E., and Zill, L. P. (1956).—*J. Biol. Chem.* **222**: 895.
Walker, N. A. (1955).—*Aust. J. Biol. Sci.* **8**: 476.
Walker, N. A. (1960).—*Aust. J. Biol. Sci.* **13**: 468.
Warburg, O., and Lüttgens, W. (1946).—*Biochimia* **11**: 303.