The Influence of the Intracellular Potential on Potassium Uptake by Beetroot Tissue

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ABSTRACT Intracellular potentials were measured in beetroot tissue during the steady-state uptake of K^+ from various solutions. In solutions containing bicarbonate, the membrane potential becomes up to 70 mv more negative than the estimated equilibrium potential for K^+. The uptake of K^+ from such solutions is correlated with variations in the potential, both when the bicarbonate concentration is changed and also when the metabolic activity of the tissue is changed by washing in water for various periods. However, the estimated permeability to K^+ varies from 0.4×10^{-7} to 1.5×10^{-7} cm·sec^{-1}. It is postulated that the change of potential arises from the metabolic transport of HCO_3^- into the cell or H^+ outwards, and that the associated uptake of K^+ is partly or entirely by passive diffusion across the cell membrane. In contrast, K^+ uptake from KCl solutions is not accompanied by any significant change in the membrane potential, which remains relatively close to the K^+ equilibrium potential. In solutions containing both $KHCO_3$ and KCl, it appears that an amount of K^+ equal to the influx of Cl^- is taken up independently of the potential, while the component of K^+ uptake which is not balanced by Cl^- uptake is related to the potential in the manner described. These results suggest that K^+ uptake is linked to Cl^- uptake in an electrically neutral active transport process.

INTRODUCTION

The diffusion of free ions across the membranes of plant cells has often been considered to play a negligible part in the metabolic accumulation of salts. This view is based on measurements of isotope exchange between the cells and the external medium. A certain fraction of ions associated with the tissue is readily exchanged within a few minutes; whereas the remainder, i.e. those which have been accumulated within the vacuole, exchange only very slowly over a period of several days (Sutcliffe, 1954; Epstein and Leggett, 1954). It is concluded that metabolic uptake occurs across a membrane which is virtually impermeable to the free diffusion of ions. This leads to the hypothesis that all ions are transported into the cell in combination with carriers, and

to the attempt to describe ion transport rates mainly by the use of Michaelis-Menten kinetics (Epstein, 1956).

This approach is criticized by Briggs (1963) who has shown that much of the kinetic data taken to support the carrier hypothesis may be just as readily explained in terms of physical diffusion. A similar view is held by Laties, MacDonald, and Dainty (1964) who interpret the kinetics of Cl^- uptake by potato tissue on the assumption that both K^+ and Cl^- diffuse passively across the cell membrane. Moreover, it now appears that the earlier data showing an apparently slow exchange of accumulated ions may have been misleading. In a more detailed study of isotope exchange in beetroot tissue, Pitman (1963) has estimated the influx and efflux at the cell membrane and vacuolar membrane separately and has shown that exchange across each membrane occurs at rates comparable with that of net salt uptake into the vacuole. Thus, given suitable gradients of electrical and chemical potential across the cell membrane, it is possible that diffusion may account for a large part of the observed rates of K^+ uptake in beetroot tissue. Recently some studies have been made of intracellular potentials in relation to uptake in other higher plants (e.g. Etherton, 1963). The present paper represents a first attempt to distinguish active and passive components of K^+ uptake in beetroot tissue on the basis of their relation to changes in the electrical potential.

METHODS

Preparation of Material

Roots of red beet (*Beta vulgaris* var. Crimson Globe) were used immediately after lifting or after storage in moist sand at 5°C. Slices 0.4 to 0.5 mm thick were cut with a bacon slicer from the widest part of the root, and disks 10 mm in diameter (consisting of parenchyma with some vascular tissue) were cut from a region about halfway along the radius of the slices. After cutting, the disks were kept normally at 20°C in vigorously aerated deionized water, changed once or twice a day. Tissue treated in this way remains healthy for more than a week, during which time its metabolic activity rises to a maximum and subsequently declines (e.g. MacDonald and DeKock, 1958).

Measurement of the Uptake of Ions

One or more days after cutting, batches of disks were blotted, weighed, and transferred to salt solutions. The disks and solution were kept in motion by a stream of air. After allowing a period of at least 3 hr for a steady rate of uptake to be established, the solutions were renewed, and the uptake (i.e., the net influx) of ions was determined by measuring the change in external concentration over a period of time. The temperature was maintained at 20 ± 1°C. The amount of tissue, volume and concentration of solution, and duration of experiment were chosen so that the change in external concentration should be measurable but not so large as to affect significantly the uptake of ions. In a typical experiment, 2 g of tissue in 100 ml of solution containing 0.6 meq/

liter K^+ absorbed 5 to 25 % of the potassium ions during a 6 hr period. Potassium was estimated by flame photometry, and chloride by potentiometric titration. Uptake was measured to an accuracy of about $\pm$ 0.05 μeq/g·hr (0.02 peq·cm^{-2}·sec^{-1}).

Changes in the internal concentration of K^+ in the tissue due to gain or loss of ions during the experiments were very small. In experiments (Fig. 4) in which uptake rates were followed over several days, the tissue was kept in deionized water, in which the loss of K^+ represented a negligible fraction of the cell contents, and new batches of tissue were transferred daily to the uptake solutions.

Measurement of Electrical Potentials

During the measurement of ion uptake, single disks were temporarily removed from the uptake solution to a Perspex perfusion chamber, where they were bathed in a similar solution, also at 20°C. As they were transferred, a fresh surface was cut with a razor to expose undamaged cells. A glass microelectrode containing 3 M KCl was inserted vertically into a cell under observation through a horizontally mounted microscope with a 40× long working distance objective. The microcapillary was connected to a calomel electrode, and the potential between this and a similar calomel electrode (without microcapillary) in the bathing solution was recorded with a Vibron electrometer and a recorder.

In order to penetrate the cellulose wall it was necessary to use thick walled microelectrodes with an outside diameter of up to 2 μ at the tip. Suitable electrodes usually showed a large tip potential in the dilute external solutions, but if the potential did not exceed -12 mv in a solution of 0.1 M KCl, the electrode was considered satisfactory. Cell potentials were then read without subtraction of the tip potential. For each experimental treatment, 10 to 20 cells were penetrated with microelectrodes. Many of these lost their contents or for some reason did not give stable potentials. Sometimes a stable potential was attained within a few seconds. Otherwise it was sometimes possible to obtain a steady high potential after moving the electrode or leaving it in the cell for up to half an hour. If the reading remained stable at its highest value for 10 min, it was accepted. If not, sealing of the electrode or leakage from the cell was suspected, and the reading was discarded.

It was often difficult to tell whether the tip of the microelectrode was in the cytoplasm or in the vacuole. Nevertheless, it will be assumed here that the recorded potentials represent the potential across the cell membrane (plasmalemma). This may be justified as follows: (*a*) It was sometimes possible to observe that the cytoplasm had accumulated around and over the tip of the microelectrode in the time taken to obtain a stable potential. (*b*) In other cases it appeared that when the electrode was inserted, a thin film of cytoplasm was stretched over its rather blunt tip so that the vacuolar membrane (tonoplast) was not penetrated. (*c*) Similar potentials were obtained whether the electrode tip penetrated only a short distance into the cell or was pushed far in. (*d*) Other work on plant cells has shown that the potential difference across the tonoplast is usually small compared with that across the plasmalemma. Etherton and Higinbotham (1960) found no significant gradient of potential across the tonoplast of *Avena* root hairs. In *Nitella translucens*, Spanswick and Williams (1964) measured a potential of 18 mv across the tonoplast, compared with 138 mv across the plasmalemma.

Measurement of Internal Ionic Concentrations

At the time of cutting the disks, blocks of parenchyma were cut from the root with a razor. These were blotted and weighed, and 1 to 2 g tissue was boiled with 100 ml deionized water for 10 min, homogenized in a further volume of water, extracted with 1 N sulfuric acid, and washed again with deionized water. The extracts were combined and K^+ and Na^+ were estimated by flame photometry, and Cl^- by potentiometric titration.

Units of K^+ Uptake

By extracting the same weights of solid tissue and of beet disks which had been washed for 1 hr to remove the contents from broken cells, the proportion of the tissue retaining its contents was found to be 65% (± about 5%) indicating an outer layer of empty cell walls about 70 μ wide. Thus the uptake by 1 g of disks represents the uptake by 0.65 g of living cells. It is desirable to express the rate of uptake in more fundamental units, and Pitman (1963) has calculated a conversion factor for beetroot tissue. Allowing for the difference in the size of the disks in the present investigation, 1 μeq/g. hr is considered to be equivalent to 0.4 peq·cm^{-2}·sec^{-1}, or 0.4 × 10^{-12}eq·cm^{-2}·sec^{-1}.

RESULTS

The Nernst Equilibrium Potential for K^+

The electrical potential at which a given ion is in diffusion equilibrium across the cell membrane is given by the Nernst equation, which for the present purpose may be written

$$E = \frac{58}{z} \log \left(\frac{C_o}{C_i} \right)$$

where E is the potential in millivolts, z is the valency (±1 in this case), and C_o, C_i, the concentrations outside and inside the cell.

The potentials measured in this investigation (which are thought to be across the cell membrane, see above) should strictly be compared with the ionic activities in the cytoplasm and the external solution. Since the cytoplasmic concentration and activity coefficient cannot be accurately estimated at present, it must suffice to take the total internal concentration. In *Nitella translucens*, the concentration of K^+ in the cytoplasm was found to be 119 meq/liter, although the concentration in the vacuole was only 75 meq/liter (Spanswick and Williams, 1964). On the other hand, the data of Pitman (1963) suggest that in beetroot the cytoplasm may have a lower concentration of K^+ than the vacuole. Taking a figure of 5% for the volume of tissue occupied by cytoplasm, Pitman's results indicate that the cytoplasmic concentration of K^+ may be around 80 to 90 meq/liter, rather than the value of 146

meq/liter found for the internal concentration in the present paper (see below). Also, the concentration of ions in the cytoplasm may vary with the experimental treatment. However, the error introduced by these uncertainties into the calculation of the K^+ equilibrium potential seems unlikely to exceed about ± 20 mv.

The internal concentrations of ions found in the beetroots used in the present investigation are given in Table I. Organic acids were not estimated, but it is likely that they comprised the bulk of the anions, as it is known (e.g. Poole and Poel, 1965) that the internal organic acid concentration can vary over a wide range to balance absorbed cations. In order to calculate a mean value for the Nernst equilibrium potential, the internal concentration of K^+ was taken as 146 meq/liter (Table I). In all experiments comparing uptake and potentials, the external K^+ concentration was set at 0.6 meq/liter.

TABLE I
ION CONCENTRATIONS IN BEETROOT TISSUE

Ion	Range of concentration	Mean
	(meq/liter)	*(meq/liter)*
K^+	127–170	146±6
Na^+	27–33	28
Cl^-	7–9	8

Using these values, the calculated K^+ equilibrium potential, E_K, is -139 mv (± 20 mv, see above).

Potentials during K^+ Uptake from Bicarbonate Solutions

Preliminary observations showed that in the presence of bicarbonate in the external solution, the membrane potential becomes up to 70 mv more negative than the estimated K^+ equilibrium potential. Such a departure from equilibrium must have a marked effect on the diffusion of K^+ ions across the membrane, tending to bring about a net uptake of potassium. In fact, the uptake of K^+ by beetroot tissue is known to increase progressively as the external bicarbonate and pH level are raised (Hurd, 1958; Poole and Poel, 1965). A series of experiments was therefore designed to compare K^+ uptake and membrane potentials in order to judge the extent to which uptake may be attributed to passive diffusion.

Table II summarizes those experiments in which uptake and potentials were measured in relation to the concentration of bicarbonate in the external solution. There is a progressive change in the potential with increasing bicarbonate concentration and pH. Moreover, the rate of K^+ uptake increases progressively as the potential diverges from its equilibrium value.

Although K^+ uptake was always found to increase with increasing bicar-

bonate concentration and pH, there was a threefold variation in the absolute rate of uptake in successive experiments. This variation is largely due to differences in the length of time during which the tissue was washed after it was cut. It is a well known feature of plant storage tissues that the activity of many metabolic processes, including salt uptake, increases with time for several days after slicing the material (e.g. MacDonald and DeKock, 1958).

The variation in metabolic activity of the material provided another means for testing the correlation between uptake and potential. For this purpose, a

TABLE II

EFFECT OF BICARBONATE ON THE MEMBRANE POTENTIAL

External solution						
$KHCO_3$	K_2SO_4	pH	Mean K^+ uptake	Mean potential	Standard error	No. of measurements
meq/liter	*meq/liter*		*peq·cm⁻²·sec⁻¹*	*mv*	*mv*	
0	0.6	5.5	(0.18)	−159.4	±4.8	17
0.1	0.5	6.8	(0.26)	−182.3	±6.1	7
0.2	0.4	7.2	(0.49)	−208.8	±5.2	10

Estimated equilibrium potential, E_K, = −139 mv.

The K^+ uptake is shown in parentheses because of a large variation in the absolute rates (see text). The relative increase in uptake with increasing bicarbonate concentration is, however, consistently observed.

constant bicarbonate level was used, and a wide range of uptake rates and potentials was obtained by washing the tissue for periods ranging from 1 day at 10°C to 9 days at 20°C. Fig. 1 shows that in this type of experiment there is a relationship between variations in K^+ uptake and changes of membrane potential. All the measurements in Fig. 1 were made at the same bicarbonate concentration (0.2 meq/liter), and the K^+ concentration was made up to 0.6 meq/liter with either K_2SO_4 (open circles) or KCl (solid circles). As in Table II, the correlation between uptake and potential is such that uptake is low at potentials close to E_K and increases as the potential becomes more negative. The relationship is not, however, that predicted by the Goldman "constant field" equation[1] (Hodgkin and Katz, 1949). The predicted relation-

[1] $$J_K = -P_K \frac{FE/RT}{1\text{-}\exp(FE/RT)} [\,[K_o] - [K_i] \exp(FE/RT)\,]$$

where

J_K = K^+ uptake (eq·cm^{-2}·sec^{-1})
P_K = K^+ permeability (cm·sec^{-1})
E = membrane potential (v)
K_o, K_i = external and internal K^+ concentrations (eq·cm^{-3})
F = the Faraday (coulombs·eq^{-1})
R = the gas constant (joules·degree absolute^{-1}·mole^{-1})
T = absolute temperature.

ship is shown by a continuous line in Fig. 1, calculated for an arbitrarily chosen value of the K^+ permeability, $P = 1 \times 10^{-7}$ cm·sec^{-1}. There are a number of possible explanations for a discrepancy of this kind. It may be that pretreatments which result in an increase in potential also increase the permeability of the membrane; there may be an interaction of K^+ ions as a result of solvent drag; or there may be an active transport process which contributes to the observed rate of K^+ uptake. Whatever the explanation, the

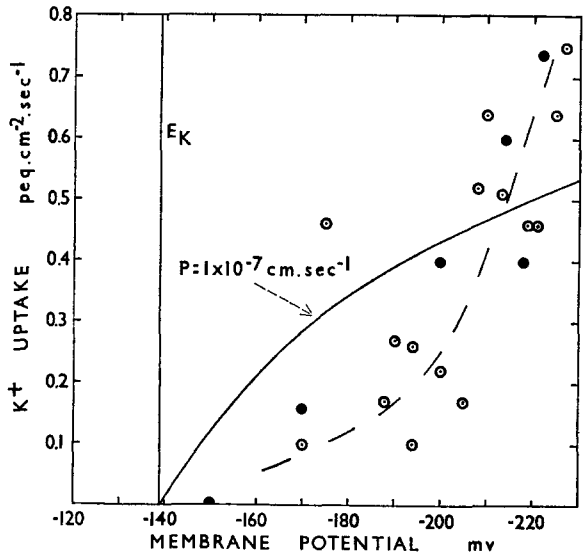

FIGURE 1. Relation between K^+ uptake rate and membrane potentials with an external solution containing 0.2 meq/liter bicarbonate, after various washing pretreatments. The external solution contained 0.2 meq/liter $KHCO_3$ plus 0.4 meq/liter K_2SO_4 (open circles) or 0.2 meq/liter $KHCO_3$ plus 0.4 meq/liter KCl (solid circles). The period of pretreatment varied from 1 day at 10°C to 9 days at 20°C. The broken line is drawn arbitrarily through the points. The solid curve shows the relation predicted by the Goldman equation for a K^+ permeability of 1×10^{-7} cm·sec^{-1}.

permeability to K^+, as estimated from the net flux, using the Goldman equation, varies in Fig. 1 from 0.4×10^{-7} cm·sec^{-1} to 1.5×10^{-7} cm·sec^{-1}. During the course of the experiments of Table II, estimates of permeability in solutions containing 0.1 meq/liter bicarbonate, and even in solutions of K_2SO_4 alone, varied within the same range.

Potentials during K^+ Uptake from KCl Solutions

In contrast to the above results in bicarbonate solutions, the membrane potential in solutions containing only KCl tends to remain relatively close to the estimated equilibrium potential for K^+. Fig. 2 shows the membrane potentials in beetroot cells with various external concentrations of KCl. The

solid line indicates the estimated value of E_K. The experimental points joined by a broken line represent the potentials measured in a single cell, with an initial external concentration of 0.1 mM KCl, as the external concentration was progressively increased, allowing time after each change of solution for the potential to reach a steady value. A number of single measurements in other cells at an external concentration of 3 mM KCl are included to show the

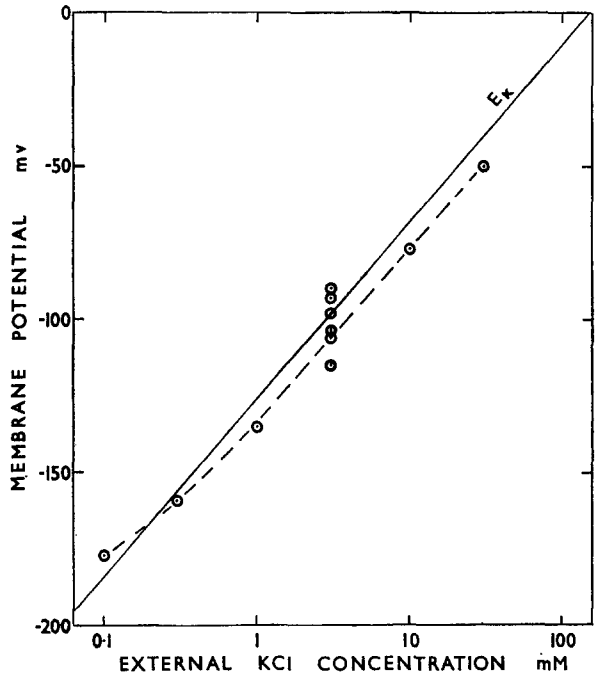

FIGURE 2. Membrane potentials in beetroot cells (after 6 to 8 days' pretreatment at 20°C) in relation to external KCl concentration. Points joined by a broken line relate to a single cell. Potentials in a number of other cells at an external concentration of 3 mM KCl are also included. The continuous line indicates the potential (E_K) at which K^+ is calculated to be in diffusion equilibrium.

usual range of error. The value of the potential in KCl solutions is probably largely determined by the diffusion of K^+ across the membrane. However, it appears that a new equilibrium is soon reached after each change of concentration, and that any driving force bringing about a continued net diffusion of K^+ across the cell membrane must be very small compared with that observed in bicarbonate solutions.

Fig. 3 shows that in KCl solutions there is no indication of a relationship between uptake rates and membrane potentials. As in Fig. 1, the differences in rate of uptake were brought about by various washing pretreatments. The results are, however, in contrast to those observed in bicarbonate solutions in

that the potential is not significantly affected by the pretreatments, and varies around a mean of −153 mv. The uptake of K^+ at various rates in the absence of any significant change in the mean potential suggests that K^+ uptake from KCl solutions occurs not by diffusion but by a metabolic process independent of the potential.

Potentials in Solutions Containing Both $KHCO_3$ and KCl

In view of the contrast between the results in solutions of KCl alone and those in solutions containing bicarbonate, it is of particular interest to study uptake

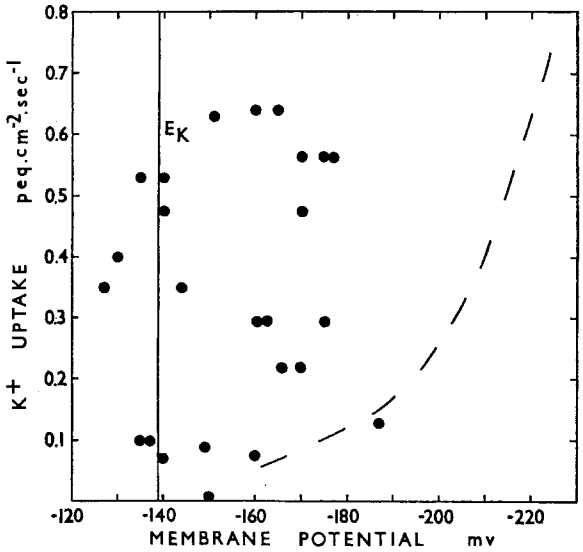

FIGURE 3. Potassium uptake and membrane potentials with an external solution of 0.6 mM KCl, after pretreatment of the tissue in water for periods of 2 to 8 days at 20°C. The broken line shows the relation found in the presence of 0.2 meq/liter bicarbonate (Fig. 1).

from solutions containing both anions. It will be shown in Fig. 4 that in such solutions the rate of K^+ uptake at any given potential seems to depend on the rate of uptake of Cl^-. In fact, K^+ uptake can apparently be divided into two distinct components: (*a*) An amount of K^+ equal to the influx of Cl^- is taken up independently of the potential. (*b*) The component of K^+ uptake which is not balanced by Cl^- uptake is related to the potential in the manner previously observed in bicarbonate solutions.

Fig. 4 shows the uptake rates of K^+ and Cl^- and membrane potentials in an external solution containing 0.1 mM $KHCO_3$ plus 0.5 mM KCl, after various periods of pretreatment in water. Increasing the duration of the pretreatment from 3 to 7 days at 20°C increased the K^+ uptake nearly threefold, the increase being balanced mainly by an increase in Cl^- uptake, with-

out any significant increase in the mean potential (lower part of Fig. 4). However, owing to the presence of bicarbonate (or increase in pH), the mean potential throughout this experiment is more negative than the potential in solutions of KCl alone (Fig. 3). This difference in potential appears to account for the excess of K^+ uptake over Cl^- uptake, with a relationship between

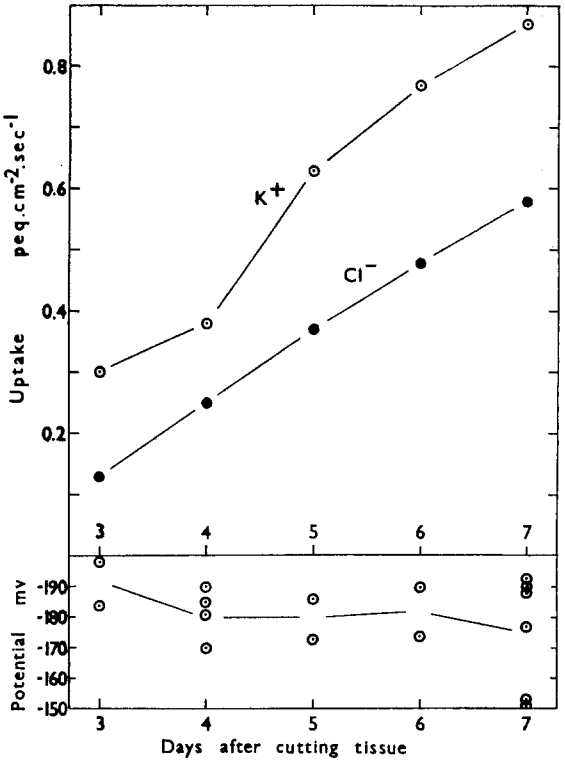

FIGURE 4. Effect of pretreatment time on the rate of uptake of K^+ and Cl^- (upper part of figure) and membrane potentials (lower part of figure) with an external solution of 0.1 meq/liter $KHCO_3$ plus 0.5 meq/liter KCl.

uptake and potential similar to that in Table II and Fig. 1. The range of permeability required to account for the excess cation uptake in Fig. 4 is 0.4×10^{-7} cm·sec^{-1} (day 3) to 0.9×10^{-7} cm·sec^{-1} (day 7), which falls within the range of permeability found in Fig. 1, where Cl^- was either absent or not absorbed to any significant extent. In fact, Cl^- uptake from solutions containing KCl in Fig. 1 balanced only 5 to 25% of the K^+ uptake, and in this case the presence of Cl^- in the external solution does not significantly increase the total K^+ uptake in relation to the potential.

It is suggested, on the basis of the above results, that the postulated metabolic uptake of K^+ from KCl solutions is closely linked with the uptake of Cl^-.

DISCUSSION

The Origin of the Membrane Potential in Bicarbonate Solutions

The uptake of K^+ from bicarbonate solutions is balanced by a quantitative decrease in the external bicarbonate concentration (Hurd, 1958). Thus electrical balance at the cell membrane is maintained by the movement of either HCO_3 inward or H^+ outward. The synthesis of organic acid which accompanies this process (Poole and Poel, 1965) presumably serves to maintain a constant internal pH level. It is not possible in this investigation to distinguish between the effects of HCO_3^- and of pH in the external solution (Poole and Poel, 1965). All bicarbonate solutions used were in equilibrium with air, and the pH varied with the HCO_3^- concentration as shown in Table II.

With the pH values and potentials in Table II, the Nernst equation shows that diffusion equilibrium for H^+ would exist across the membrane at an internal pH of about 3.7. If there is no appreciable gradient of CO_2 concentration, the ratio of bicarbonate ions across the membrane will be the inverse of the ratio of H^+ ions, and diffusion equilibrium for HCO_3^- would also exist at an internal pH of about 3.7. If the internal CO_2 level is higher, diffusion equilibrium for HCO_3^- would require a lower internal pH. A net movement of H^+ outward or of HCO_3^- inward could result from passive diffusion only if the cytoplasmic pH were lower than that calculated for equilibrium, i.e. lower than pH 3.7, which seems unlikely. It is postulated, therefore, that the influx of bicarbonate ions or efflux of hydrogen ions which accompanies K^+ uptake from bicarbonate solutions is brought about by a metabolic transport process. The correlation between uptake and potential in bicarbonate solutions suggests that it is this transport of HCO_3^- or H^+ which gives rise to the negative potentials observed. This hypothesis implies that the transport mechanism carries a net charge, and corresponds with the idea that K^+ ions are not actively transported together with HCO_3^- (or in exchange for H^+) on a one to one basis, but move at least partly by diffusion in response to the gradient of electrochemical potential across the membrane.

The Passive Permeability to K^+

Although it is shown that the change of potential in bicarbonate solutions does provide a driving force for the diffusion of K^+ into the cell, an independent estimate of the permeability to K^+ is required to show whether the rate of diffusion is adequate to account for the observed rates of uptake. Such an estimate is provided by measurements of K^{42} exchange in beetroot tissue by Pitman (1963). Calculations using Pitman's results for the efflux of K^+ across the cell membrane at 25°C with an external solution of 5 meq/liter KCl, assuming an electrical potential close to its equilibrium value, give a

permeability coefficient of 0.2×10^{-7} cm·sec^{-1} to 0.4×10^{-7} cm·sec^{-1}. The range of permeability required to account for the uptake of K^+ from bicarbonate solutions in the present investigation is 0.4×10^{-7} cm·sec^{-1} to 1.5×10^{-7} cm·sec^{-1} (Fig. 1). The agreement with the isotope fluxes within an order of magnitude is satisfactory considering the approximations involved. However, more data on isotope fluxes are required to investigate the apparent variation of permeability in Fig. 1.

Transport of K^+ and Cl^- across the Cell Membrane

The above estimates of the K^+ permeability in beetroot are too low to account for the uptake of K^+ from KCl solutions with the potentials which are observed, and it is postulated that K^+ uptake from KCl involves a metabolic transport across the cell membrane. It is also shown, in Figs. 3 and 4, that when K^+ uptake is accompanied by Cl^- uptake, its rate may vary over a wide range without any significant change in the potential. This suggests that K^+ uptake may be effected by an electrically neutral active transport mechanism, in which K^+ and Cl^- ions are carried simultaneously across the membrane, perhaps in combination with a single carrier molecule. The first example of a neutral cation-anion pump (in this case of NaCl) was demonstrated in the gall bladder by Diamond (1962). Two other observations lend support to such a mechanism for KCl transport in beetroot. First, Cl^- uptake is not depressed in the presence of bicarbonate (Hurd, 1958; Poole and Poel, 1965). Thus Cl^- appears to be taken up by a mechanism independent of the membrane potential. Second, it has been found in other studies of salt uptake by beetroot tissue that variations in Cl^- uptake from KCl solutions are closely paralleled by changes in the rate of K^+ uptake (Dale and Sutcliffe, 1959; Pitman, 1964).

Pitman (1964) has suggested that in beetroot tissue both K^+ and Cl^- ions diffuse passively across the cell membrane, and that the ions interact through their dependence on the membrane potential. The present work provides the first detailed measurements of membrane potentials in beetroot, and although further information is required about certain other factors such as the cytoplasmic concentration of K^+, it now appears unlikely that KCl uptake can be explained by Pitman's hypothesis. As shown above, the simplest interpretation of the present results is in terms of a linked active transport of K^+ and Cl^- across the cell membrane.

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