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THE INFLUX OF POTASSIUM INTO *CHLORELLA PYRENOIDOSA*

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SUMMARY

1. Using radiotracer it has been shown that under steady-state conditions the majority of the intracellular K^+ in Chlorella exchanges by first-order kinetics.

2. The unidirectional influx of K^+, in the absence of net movement, is approximately 1 pmole K^+ per $sec \cdot cm^2$ in the light but in the dark is reduced to 0.18 pmole K^+ per $sec \cdot cm^2$.

3. The influx rates, particularly for illuminated cells, were temperature sensitive and seem to be under metabolic control as expected for an active process.

4. The experiments suggest that the light-induced transport mechanism is independent of net carbon fixation and may be utilizing energy derived directly from electron transport processes.

5. An estimate of the passive permeability coefficient for K^+ movement into illuminated Chlorella cells gave a value of $2.0 \cdot 10^{-8}$ $cm \cdot sec^{-1}$.

INTRODUCTION

Although in many respects microorganisms are excellent materials for studying ion transport mechanisms, to date very few systems have been thoroughly investigated. However, current interest concerning light-stimulated ion movement, both in chloroplasts and intact cells, suggested that a photosynthetic microorganism such as Chlorella would be suitable for further studies on this aspect of cell metabolism.

Like many other plant and animal cells, the major intracellular cation in Chlorella is K^+. This was originally reported some years ago by SCOTT[1] who demonstrated that as long as there was sufficient K^+ available in the bathing medium this cation is accumulated in preference to Na^+. Since then COHEN[2,3] has presented evidence that this accumulation is brought about by specific carriers situated on the cell surface and obtained chemical and dimensional information about the nature of the binding sites. Recently a method of inserting microelectrodes into this organism has been described[4] and the measured potential difference correlated with the ionic distribution. These results were consistent with the earlier findings and indicated that active transport mechanisms were most probably responsible for the uptake of K^+

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea.

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and extrusion of Na^+. In addition radiotracer studies demonstrated further the likelihood of a Na^+ efflux pump in this alga[5].

This paper reports experiments designed to measure the magnitude and kinetics of the steady-state influx of K^+ into Chlorella and to investigate the photosensitivity of this uptake.

MATERIALS AND METHODS

The alga, *Chlorella pyrenoidosa*, was an Emerson strain for which the culturing and harvesting procedures have already been given[4]. After centrifugation the cell pellets were washed and suspended in culture solution which served as the experimental medium except where stated. The number of cells associated with these standard suspensions were determined by counting either with a haemocytometer or a Coulter counter (Model A).

The suspensions were contained in glass vessels, some of which were covered in black tape, placed in a constant temperature bath and illuminated at 500 ft-candles by two 150-W incandescent spot lights. The cells were allowed to stand in these reaction vessels for 20–30 min before tracer was added and for those experiments involving inhibitors this period served as the pretreatment time. The cells were kept in suspension by bubbling with moist air which in some cases was replaced by other gases. The time course of tracer uptake was followed by withdrawing 1-ml aliquots, filtering and assaying internal radioactivity as previously described[5]. The tracer was ^{42}K obtained either as the chloride from the Radiochemical Centre, Amersham, or as the spectroscopically pure carbonate from the Atomic Energy Establishment, Harwell.

Oxygen measurements were made with a Beckman 325814 macro electrode. Determinations were carried out on cells suspended in culture medium which had been equilibrated with 5 % CO_2 *plus* 95 % air. Variation in oxygen tension was detected on a Vibron electrometer (Model 33B2) as a potential change across a resistor and registered on a Bausch–Lomb chart recorder.

RESULTS

Estimate of surface area

Measurement of cell numbers gave that 1 ml of packed cells contained approx. $5 \cdot 10^9$ cells. Combining this with the previously reported true cellular volume associated with the same quantity of packed cells[4] gives a calculated mean diameter of 6.4 μ. This estimated value compares very well with the optically measured diameters which averaged out at 6.0 μ. Thus, if 6.2 μ is taken as the typical diameter then the surface area of 1 ml of packed cells is $6.0 \cdot 10^3$ cm^2.

Kinetics and magnitude of K^+ influx

If it is assumed that the cells and the external medium act as a steady-state two compartment system then the internal radioactivity A_i in counts/min at time t after addition of tracer is given by $A_i = A_\alpha (1 - e^{-kt})$ where A_α is the internal activity at isotopic equilibrium and k is a rate constant in reciprocal time units. According to this first-order equation a plot of $\log A_\alpha/(A_\alpha - A_i)$ against time gives a straight line.

As shown in Fig. 1a such a relationship was found both for light and dark treated cells. The value of A_α was determined from the illuminated cells when they had reached isotopic equilibrium and represented an internal concentration corresponding to flame photometry measurements[4].

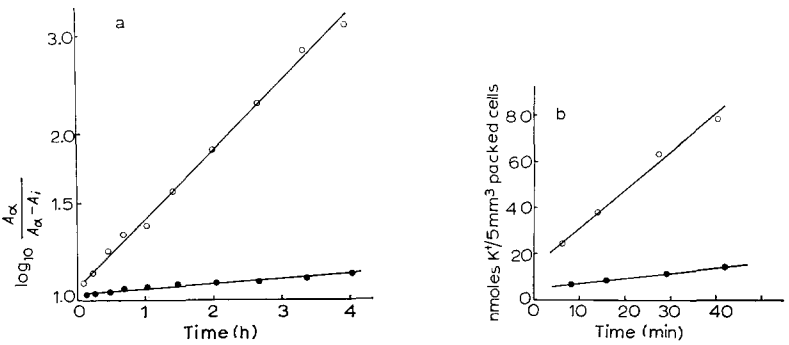

Fig. 1. a. A plot of $\log A_\alpha/(A_\alpha - A_1)$ against time for a long-term uptake of ^{42}K by light (○) and dark (●) treated cells. b. A short-term uptake of ^{42}K showing the initial rates for light (○) and dark (●) conditions.

The slopes of these semilog plots can be used to calculate the uptake rates, but the rather long duration of the experiments made it more convenient, in the majority of cases, to measure the initial influx of ^{42}K as shown in Fig. 1b. The rates of K^+ influx (moles/sec·5 mm^3 packed cells) were obtained from the slopes of these initial time courses or alternatively calculated using the expression $M = A_1/S_0at$ where M is influx in moles·cm^{-2}·sec^{-1}, A_1 internal activity in counts/min per cm^3 of cellular volume, a is surface area in cm^2 and S_0 is the external specific activity. In Table I the mean light and dark uptake rates, under fixed external conditions and in the absence of net K^+ movement, are listed.

Although in the light the majority of the cellular K^+ exchanged by first-order kinetics there was a small component, representing about 1–2 % of the total internal K^+, which exchanged much faster. The uptake of ^{42}K into this smaller compartment could be followed by rapid sampling over the first few minutes of tracer influx as shown in Fig. 2. Moreover, this initial phase was not readily removed since washing each aliquot with 20 mM $CaCl_2$ for 30 sec, instead of the usual distilled water wash, did not reduce its magnitude.

TABLE I

LIGHT AND DARK INFLUX RATES OF POTASSIUM INTO *Chlorella pyrenoidosa*

The cells were suspended in culture medium (pH 6.8) at a temperature of 25 ± 0.5°. The intensity of the light at the reaction vessels was about 500 ft-candles. The results are quoted ± S.E. with the number of experiments on which the mean is based given in parenthesis.

Conditions	*K^+ influx rate*	
	pmoles/sec·5 mm^3 packed cells	*pmoles·cm^{-2}·sec^{-1}*
Light	19.1 ± 1.0 (31)	1.03 ± 0.10
Dark	3.4 ± 0.1 (19)	0.18 ± 0.01

Effect of changing the external K^+ level

The influx rates given in Table I were measured for cells suspended in culture medium with an external K^+ concentration of 6.5 mM. At this value the light rate was independent of the external K^+ level and not until the concentration of this

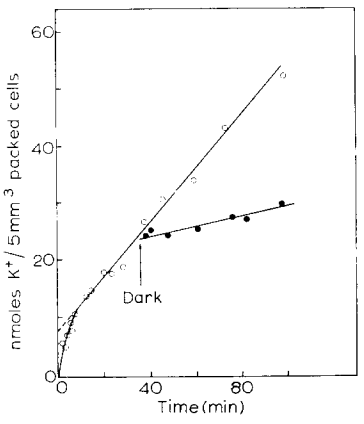

Fig. 2. A time course of K^+ influx showing the effect of plunging some of the illuminated cells (○) into the dark (●). The plot also shows rapid sampling over the first few minutes of the uptake.

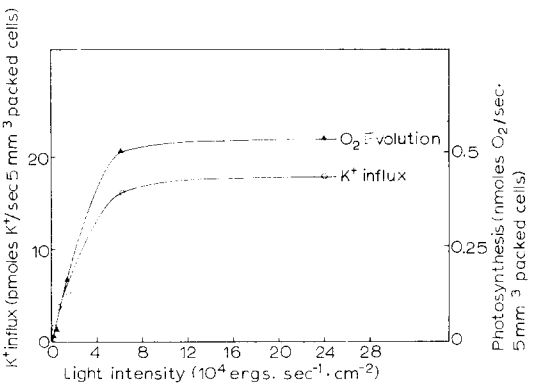

Fig. 3. Rate of influx of K^+ (○) and photosynthesis (▲) as a function of white light intensity. The K^+ influx values represent the light-stimulated portion, that is, the dark rates have been subtracted.

cation was below 0.5 mM did the influx rate fall off. For example, in an experiment on illuminated cells suspended only in KCl solution at various concentrations, the maximum rate occurred at 0.4 mM with a Michaelis constant of approx. 0.07 mM.

The action of light

From Table I it can be seen that the action of light was to stimulate the influx of K^+ and this sensitivity is clearly shown in Figs. 1 and 2. In the case of Fig. 2 half the suspension was plunged into the dark which resulted in an immediate establishment of the dark uptake rate. However, when the dark treated cells were suddenly exposed to illumination a small rapid transient uptake was observed before the usual steady-state rate was obtained.

The sensitivity of the influx to various light intensities is shown in Fig. 3 and compared with the rate of photosynthetic oxygen evolution under exactly similar lighting conditions. It can be seen that both processes saturated at about the same light intensity.

Temperature sensitivity

As shown in Fig. 4 both the light and dark uptakes were sensitive to temperature. The light rate was particularly affected by these 10° changes and approximately corresponded to the variation of O_2 evolution under the same conditions. At 5° there was virtually no O_2 turnover suggesting that the light and dark influxes of K^+ at this temperature represents the passive components of the uptakes.

Effect of various gassing conditions

The effect of various gaseous atmospheres on the uptake rates of K^+ is shown in Fig. 5. Although CO_2-free air and nitrogen caused a reduction in the photosensitive K^+ influx, the rates were still maintained above the dark level. In the absence of CO_2 no net carbon fixation can occur, indicating that the light-promoted uptake of K^+ by Chlorella is geared more closely to the primary redox changes of photosynthesis.

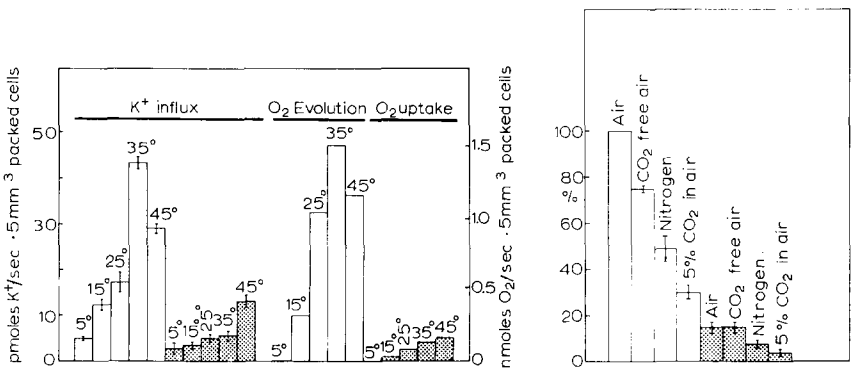

Fig. 4. The sensitivity of light (unshaded) and dark (shaded) K^+ influxes, photosynthesis and respiration to temperature changes from 5° to 45°. Tracer and oxygen measurements were not carried out on the same suspensions. S.E. of the regression lines are included for the K^+ influxes.

Fig. 5. The effect of various gaseous atmospheres on the K^+ influx for light (unshaded) and dark (shaded) conditions. The results are presented as the percentage of the light controls and are the mean of 3–5 experiments. The S.E. are shown for each condition.

Under conditions of no carbon fixation only pseudocyclic and cyclic electron flow are possible. Thus, it would seem that the light-stimulated uptake of K^+ under N_2 could be supported by cyclic electron flow while in the presence of CO_2-free air, oxygen may act as a terminal electron acceptor and pseudocyclic flow may then also partially support the K^+ uptake. The inhibitory effect of the higher levels of CO_2 emphasises further the unlikelihood that this light-sensitive influx of K^+ into Chlorella is associated with photosynthetic carbon metabolism.

The decrease of the dark rate in the presence of N_2 suggests that there may be some requirement for respiration although the further inhibition by 5 % CO_2 is more difficult to explain.

The effect of carbonyl cyanide m-chlorophenylhydrazone

The possibility that the light-stimulated K^+ influx is supported by non-cyclic and cyclic electron flow in this alga, suggested that the associated phosphorylation processes may be responsible for the enhanced uptake. In Fig. 6 the action of carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), an effective uncoupler of photophosphorylation, can be seen. In the presence of this compound the light rate was reduced to 70 % with $5 \cdot 10^{-6}$ M and almost to the dark level with 10^{-5} M CCCP.

Addition of exogenous sugars

To test if the K^+ uptake rates were sensitive to possible changes in available ATP levels within the cells, the effect of adding exogenous glucose was investigated.

It can be seen in Fig. 7 that the addition of 10^{-2} M glucose to the suspension inhibited the normal light rate while stimulating the dark uptake. Since it has been suggested that the photoassimilation of glucose by Chlorella requires the consumption of ATP[6], the observed inhibition may reflect a competition between the two accumulation processes for this high-energy compound. On the other hand, addition of glucose to dark treated cells is known to enhance respiration in this alga[7] and in so doing may stimulate the uptake rate by increasing the ATP available to the K^+ transport sites.

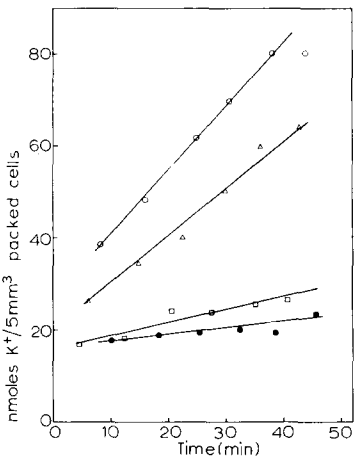

Fig. 6. The time course of K^+ influx under light (○) and dark (●) conditions and the effect of $5 \cdot 10^{-6}$ M CCCP (△) and 10^{-5} M CCCP (□) on the light rate.

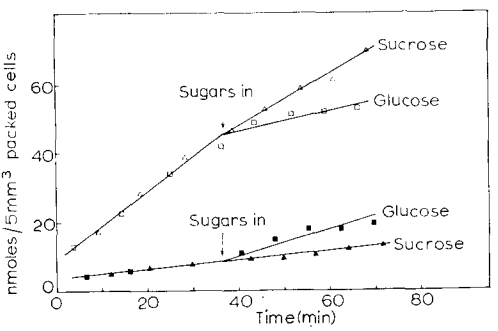

Fig. 7. The effect of "dumping in" 10^{-2} M glucose (■, □) and 10^{-2} M sucrose (▲, △) on the time course of K^+ uptake both for light (△, □) and dark (▲, ■) treated cells.

Sucrose was added as a control because it was considered that this sugar would not be involved in similar phosphorylation reactions as suggested for glucose. This was probably not entirely correct since there is evidence that sucrose can enter Chlorella cells[8] and may explain the slight decrease of the light rate when this sugar was added.

The effect of strophanthidin

The active influx of K^+ into many cells is linked to Na^+ efflux and it is usual for this exchange pump to be specifically inhibited by cardiac glycosides[9]. With this in mind, the light-stimulated uptake of K^+ by Chlorella was measured in the presence of the low molecular weight cardiac glycoside, strophanthidin. However, with $5 \cdot 10^{-4}$ M and 10^{-4} M no detectable reduction in the influx rate was observed.

DISCUSSION

It has been shown that under steady-state conditions the exchange of the majority of internal K^+ in illuminated Chlorella cells is governed by a single rate constant. Such simple kinetics however, do not constitute evidence for a simple structural model. Indeed, although these cells are non-vacuolated they contain many

membraneous organelles which may act as additional compartments. Nevertheless, the kinetics suggest that they have permeabilities which are higher to K^+ than the main diffusion barrier. The results are therefore consistent with the hypothesis that the rate of exchange is governed by a surface membrane and that this cation probably exists in free solution within the cells. In addition, the uptake rate of K^+ in the light of about 1 pmole·cm^{-2}·sec^{-1} is very similar to the K^+ fluxes across the plasma membrane of other cells[10–13].

The faster uptake representing only 1–2 % of the total internal K^+ was initially thought to arise from fixed negative charges associated with the cell wall of this alga. This assumption was most likely incorrect since when the sampling aliquots were washed with ionic solutions, instead of distilled water, the component was not readily removed. Also, the exchange times were rather slower than expected for the walls of these small spherical cells even if some limitations were imposed by unstirred layers. In agreement with this is the chemical analysis of the walls of Chlorella by NORTHCOTE, GOULDING AND HORNE[14]. They found this alga to contain no polyuronic acids whose carboxylic groups are thought to be responsible for the high concentrations of negative charges found in the walls of some plant cells[15]. Thus, the nature of this initial uptake remains obscure. Since the experiments were conducted on heterogeneous cell populations the possibility arises that this component represents a small proportion of the cells in any particular suspension and does not necessarily imply that each cell contains an additional small compartment.

The metabolic sensitivity of the K^+ uptake, particularly in the light, is in agreement with the previously reported electrochemical potential analysis which had suggested that this alga actively accumulates K^+.

For the light-promoted uptake the results indicate that the required energy is derived from photosynthetic processes. However, there was no evidence to link this accumulation with carbon metabolism resulting from CO_2 fixation and, indeed, it is most likely that the uptake mechanism is utilizing energy derived from electron transport. It seems that both pseudocyclic and cyclic electron flow can partially support the photo-induced uptake and experiments with antimycin A, presented elsewhere[16], have shown both the anaerobic and 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU)-resistant light-supported K^+ influxes to be reduced further by this poison. The inhibitory effect of the uncoupler CCCP suggests that it is the phosphorylation processes linked with electron transport which are responsible for the uptake of this cation. A part of the dark influx may also require metabolic energy but in this case the ATP would presumably result from oxidative phosphorylation. The effect of exogenous glucose is consistent with this and suggests further that the light and dark rates are governed by the level of ATP at the transport sites.

Thus, it would seem that Chlorella, under these experimental conditions, possesses an ATP requiring mechanism for actively accumulating K^+. The specific K^+ sites studied by COHEN[2,3], which were believed to be associated with a surface membrane, may be the carriers responsible for this process. Certainly the independence of the uptake rate to changes of external K^+ above a certain level would suggest the presence of only a limited number of carriers. However, interpretation of saturation kinetics for the influx of this cation as a function of the external concentration, is complicated by the possible changes of membrane potential[4].

An active K^+ influx pump is a familiar feature of many types of cells and has

been suggested for photosynthetic algae[17–20] as well as for other microorganisms[21,22]. In some of these algae this mechanism is light-sensitive[17,18] and, in particular, studies on *Nitella translucens*[23] and *Hydrodictyon africanum*[24] have also implicated ATP derived from photosynthetic electron flow as the required energy source. In addition it was suggested that the active K^+ influx into these two coenocytes is linked to the Na^+ extrusion pump in a manner similar to that found for many animal cells. However, such a closely coupled exchange system seems unlikely in Chlorella as indicated by the large difference in the magnitude of the two flux rates[5] and the lack of inhibition observed with cardiac glycosides.

In the case of microorganisms the presence of an active K^+ pump is almost always implied although detailed electrochemical potential measurements are usually lacking. However, ROTHSTEIN[21] has speculated about the function of this accumulation, believing that its purpose is to produce a high internal hypertonicity essential for growth and expansion of these walled cells. His arguments require that those microorganisms living in dilute solutions possess a relatively impermeable membrane such as to reduce the back leakage of this cation down the thermodynamic gradient. Since the membrane potential has been measured for illuminated Chlorella cells bathed in culture medium[4], it is possible to estimate a rough value of the passive permeability coefficient for K^+ movement in this organism. This quantity can be calculated for unidirectional tracer influx from the constant-field theory of GOLDMAN[25] using the following equation[26]:

$$M = -\frac{ZFE}{RT} \cdot P_K \cdot \frac{[K^+]_0}{(1 - \exp ZFE/RT)}$$

where M is the passive influx of K^+ in moles·cm^{-2}·sec^{-1}, P_K is the permeability coefficient in cm/sec, E is the membrane potential, $[K^+]_0$ is the external K^+ concentration and the other symbols have their usual meanings. Evidence that a proportion of the light-stimulated K^+ influx contains a passive component comes from the work at low temperature and with inhibitors[16], but there is some uncertainty of its exact magnitude. However, if it is assumed that 25% of the light rate is passive then the application of the above equation gives $P_K = 2.0 \cdot 10^{-8}$ cm/sec. This value is in agreement with the above speculations[21] since it suggests that Chlorella possesses a rate-limiting membrane which is at least 10 times less permeable to K^+ than the outer cytoplasmic membranes of vacuolated algae[11,13,27].

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REFERENCES

1 G. T. SCOTT, *J. Cellular Comp. Physiol.*, 21 (1943) 327.
2 D. COHEN, *J. Gen. Physiol.*, 45 (1962) 959.
3 D. COHEN, *J. Gen. Physiol.*, 45 (1962) 979.

4 J. BARBER, *Biochim. Biophys. Acta*, 150 (1968) 618.
5 J. BARBER, *Biochim. Biophys. Acta*, 150 (1968) 730.
6 O. KANDLER, *Z. Naturforsch.*, 9b (1954) 625.
7 J. MYERS, *J. Gen. Physiol.*, 30 (1947) 217.
8 R. T. WEDDING, L. C. ERICKSON AND M. KAY, *Plant Physiol.*, 34 (1959) 3.
9 P. F. BAKER, *Endeavour*, 15 (1966) 166.
10 W. EPSTEIN AND S. G. SCHULTZ, *J. Gen. Physiol.*, 49 (1966) 469.
11 E. A. C. MACROBBIE, *J. Gen. Physiol.*, 45 (1962) 861.
12 A. B. HOPE, *Australian J. Biol. Sci.*, 16 (1963) 429.
13 J. A. RAVEN, *J. Gen. Physiol.*, 50 (1967) 1607.
14 D. H. NORTHCOTE, K. J. GOULDING AND R. W. HORNE, *Biochem. J.*, 70 (1958) 391.
15 J. DAINTY, A. B. HOPE AND C. DENBY, *Australian J. Biol. Sci.*, 13 (1960) 267.
16 J. BARBER, *Nature*, 217 (1968) 876.
17 E. A. C. MACROBBIE AND J. DAINTY, *Physiol. Plantarum*, 11 (1958) 782.
18 J. GUTKNECHT, *Biol. Bull.*, 129 (1965) 495.
19 J. GUTKNECHT, *Biol. Bull.*, 130 (1966) 331.
20 W. A. DODD, M. G. PITMAN AND K. R. WEST, *Australian J. Biol. Sci.*, 19 (1966) 341.
21 A. ROTHSTEIN in J. F. HOFFMAN, *The Cellular Function of Membrane Transport*, Prentice-Hall, New Jersey, 1964, p. 23.
22 C. W. SLAYMAN AND E. L. TATUM, *Biochim. Biophys. Acta*, 102 (1965) 149.
23 E. A. C. MACROBBIE, *Biochim. Biophys. Acta*, 94 (1965) 64.
24 J. A. RAVEN, *J. Gen. Physiol.*, 50 (1967) 1627.
25 D. E. GOLDMAN, *J. Gen. Physiol.*, 27 (1943) 37.
26 G. E. BRIGGS, A. B. HOPE AND R. N. ROBERTSON, *Electrolytes and Plant Cells*, Blackwell, Oxford, 1961, p. 19.
27 J. DAINTY, *Ann. Rev. Plant Physiol.*, 13 (1962) 379.