Potassium Transport in Non-growing Mycelium of *Neocosmospora vasinfecta*

By K. BUDD

Department of Biology, Queen's University, Kingston, Ontario, Canada

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SUMMARY

Mycelium of potassium-limited *Neocosmospora vasinfecta* had a lowered potassium content and an increased sodium content compared with mycelium grown in presence of excess potassium and no sodium. Potassium uptake involves the exchange for Na^+ and probably also for H^+ .

Total potassium uptake was decreased to half by a fivefold equivalent excess of rubidium, but was essentially unaffected by a 100-fold equivalent excess of sodium. Calcium and magnesium had intermediate effects. Potassium uptake was inhibited by sodium azide (mM) and by dinitrophenol (0.3 mM). These depressed the equilibrium potassium level but had a much smaller effect on the time required to reach equilibrium. Loss of potassium by K^+ -loaded mycelium to sodium azide or dinitrophenol solutions or to water was small. Much greater losses occurred to solutions of rubidium but not of sodium.

It is suggested that the equilibrium potassium level is determined only in part by exchange-diffusion at the mycelium surface, and that net potassium uptake is a metabolic process.

INTRODUCTION

Studies of the transport of inorganic ions by mycelial fungi are scarce. The only fungus which has been systematically studied from this point of view is the yeast, *Saccharomyces cerevisiae* (Rothstein, 1965). Although some of the major features of ion transport in this fungus are now clear, it seems impossible to decide whether yeast is representative of the fungi as a group in this respect.

In the absence of growth, net movements of ions between the cells and the environmental fluid can be observed, under suitable conditions, with yeasts and other micro-organisms. The use of non-growing organisms is an advantage, since it simplifies interpretation of the relationship of metabolism to the transport processes, and allows the latter to be studied in relatively simple experimental solutions. In preliminary experiments, mycelium of *Neocosmospora vasinfecta* grown in a medium of high (106 m-equiv./l.) potassium content (Budd, 1969*a*) was harvested in mid-logarithmic phase and transferred to solutions of potassium salts lacking a nitrogen source. In such solutions, growth as defined by net protein increase was not measurable. Net uptake of potassium by this mycelium was small and variable. Replacement of most of the K^+ of the growth medium by Na^+ produced a mycelium of relatively low K^+ content when harvested in late logarithmic phase. Some of the characteristics of potassium transport by this low-potassium mycelium are reported in this communication. A preliminary report of this work has already been published (Budd, 1969*b*).

METHODS

Neocosmospora vasinfecta ATCC 11686 (American Type Culture Collection, Rockville, Md., U.S.A.) was grown in liquid shaken cultures from standardized mycelial inocula for 46 to 50 hr at $25 \pm 0.5^\circ$. The culture medium was a low- K^+ variant of the medium G1 previously described by Budd (1969*a*) and contained (per l. glass-distilled water): glucose, 30.0 g.; $NaNO_3$, 8.5 g.; $MgSO_4 \cdot 7H_2O$, 2.5 g.; KH_2PO_4 , 0.10 g.; $NaH_2PO_4 \cdot H_2O$, 0.90 g.; trace elements according to Horowitz & Beadle (1943); pH before autoclaving, 4.5. Analysis showed the K^+ and Na^+ contents of this medium to be 0.7 and 106.8 m-equiv./l. respectively, in contrast to 106.0 m-equiv./l., and essentially zero for medium G1. Growth on the low- K^+ medium was logarithmic from the time of inoculation to the 56th hr of incubation. The mycelium was separated by filtration, washed by suspension in distilled water, and samples of 30 to 50 mg. dry wt were prepared as described by Budd & Harley (1962).

Incubation was at room temperature (22.5 to 25.5°) with aeration (Budd, 1969*a*), terminated by rapid filtration on Whatman No. 54 paper circles. After about 10 sec. rinsing with glass-distilled water on the filter the mycelium was extracted, usually with 1.0 N- HNO_3 , for 24 hr in a centrifuge tube. Mycelium and extractant were then separated by centrifugation, the mycelium extracted with more 1.0 N- HNO_3 and the combined extracts made to known volume with glass-distilled water. In some experiments this procedure was replaced by wet combustion of the sample with 1 ml. 70% (w/v) HNO_3 , followed by dilution to known volume with glass-distilled water. Extraction of sodium and potassium by these two procedures agreed to within $\pm 2\%$.

Sodium and potassium contents of suitably diluted portions of the mycelial extracts were determined by flame emission spectrophotometry, with a Unicam SP 90 A atomic absorption spectrophotometer in the emission mode. Standard solutions and blanks were adjusted to the same content of nitric acid as the unknown solutions. Mutual interference between sodium and potassium in flame emission analysis was negligible at their relative concentrations in the extracts. Rubidium did interfere with this method of potassium estimation. A correction factor was established by first determining the rubidium concentration of the extract as described below, and then determining the expected error in potassium flame-emission from experimentally prepared curves which related this emission to Rb^+ and K^+ concentrations in mixed solution.

Rubidium uptake was determined by using ^{86}Rb as a tracer. The isotope was obtained from the Radiochemical Centre, Amersham, Buckinghamshire, England as an aqueous solution of $RbCl$; this was diluted immediately before use with carrier $RbCl$ to approximately $2.5 \mu Ci/m\text{-equiv. } Rb^+$. The amount of isotope in mycelial extracts was determined by liquid scintillation spectrometry with the Packard '3002' Tri-Carb system and the second solution of Bruno & Christian (1961) as liquid fluor. The specific activity of the $RbCl$ supplied to the mycelium was determined in the same way at the time of assaying the extracts.

Chloride in mycelial extracts was determined by coulometric titration with a Buchler-Cotlove automatic chloride titrator. All pH measurements were made with a Heathkit model EUW-301 recording pH meter and a Sargent combination electrode.

Ouabain octahydrate was purchased from Sigma Chemical Co., St. Louis, Mo.,

U.S.A. and rubidium chloride from Alfa Inorganics, Inc., Beverly, Mass., U.S.A. All other chemicals were of analytical grade.

RESULTS

Table 1 shows the levels of sodium and potassium in mycelium grown in low-K⁺ medium and in normal mycelium from G1 medium. Growth on the low-K⁺ medium resulted in a significant decrease in potassium content, and an increase in that of sodium. The specific growth rates in the two media were identical for at least 56 hr (to a yield of about 2.5 g. dry matter/l.). It is clear that the levels of K⁺ and Na⁺ could be manipulated within the limits of these experiments without fundamentally changing the metabolic characteristics of the mycelium.

Table 1. *Sodium and potassium contents of normal and low-K⁺ mycelium of Neocosmospora vasinfecta*

Mycelium	Sodium			Potassium		
	Mean*	σ	No.	Mean	σ	No.
Normal	0.98	0.35	9	62.08	8.39	12
Low-K ⁺	26.26	9.06	16	21.70	6.52	16

* Values are μ -equiv./100 mg. dry weight. σ = Standard deviation. No. = number of experiments. Growth conditions as in text.

On transfer to dilute solutions of potassium salts, the low-K⁺ mycelium took up potassium and lost sodium, reaching new equilibrium levels of both ions after 2 to 3 hr. Figure 1 shows the results of an experiment in which KCl (0.1 mM) was the incubation medium. The pH value was monitored continuously; the changes recorded are characteristic and differed only slightly for more concentrated KCl solutions. Changes in pH value of this order had essentially no effect on the net K⁺ uptake (Fig. 2). As shown in Fig. 1, potassium uptake and sodium loss were roughly equivalent; after 3 hr 5.1 μ -equiv. K⁺ were absorbed for every 4.2 μ -equiv. Na⁺ lost. However, results to be presented showed that the K⁺:Na⁺ transport ratio had no characteristic value; it was strongly affected by experimental conditions, and was not usually close to unity. It also appears unlikely that deviations of the K⁺:Na⁺ ratio from unity were closely related to the amounts of chloride absorbed, because chloride uptake was consistently small during the first hour, the period when the greatest adjustment in K⁺ and Na⁺ levels took place (Fig. 1).

Potassium uptake was essentially complete after 2 hr for KCl concentrations between 0.1 μ M and 10 mM. The relationships of K⁺ uptake and Na⁺ loss to KCl concentration for a 2 hr incubation period are shown (Fig. 3). The total potassium uptake varied somewhat less than twofold over this 10,000-fold range of K⁺ concentration, while Na⁺ loss was unaffected.

Glucose (0.1%) in the medium had no effect on the uptake of K⁺ or loss of Na⁺ during 2 hr.

Effects of Inhibitors. Sodium azide and 2,4-dinitrophenol (DNP) strongly decreased the K⁺ level at 2 hr but they had a smaller effect on the time required to reach equilibrium, as shown for sodium azide at 1 mM in Fig. 4. The effects of DNP concentrations

are shown in Table 2. The total uptake of K^+ could be decreased by 70 to 80% with the proper concentration of inhibitor. On the other hand, Na^+ loss accompanying K^+ uptake was much less sensitive to both inhibitors. Consequently, the $K^+ : Na^+$ transport ratio fluctuated, e.g. from 2.3 to 1.0 in Table 2.

Fig. 1. Changes in *N. vasinfesta* mycelial ion levels and in extracellular pH values, during incubation of low- K^+ mycelium in 0.1 mM KCl at 25.5° (The pH values are those measured a sample size of 38 mg. dry wt incubated in 1 l. KCl.) Bars show range of duplicates.

Fig. 2. Effect of pH value on potassium uptake from mM- K^+ by *N. vasinfesta*. Uptake for 2 hr at 23.5°. Buffer system below pH 5.0, KH phthalate + HCl; above pH 5.0, KH_2PO_4 + KOH. Vertical bars show range of duplicates; horizontal bars connect initial and final pH values.

Ouabain (0.1 mM) had no effect on the movement of either K^+ or Na^+ when presented simultaneously with the KCl. When the KCl was presented following pre-incubation 1 hr with ouabain, a 15% decrease in K^+ transport and no significant effect on Na^+ transport were observed over 2 hr.

Effect of other cations. Sodium and magnesium were the major cations in the growth medium used in these experiments. Of these, Na^+ had no significant effect on the 2 hr

Table 2. Effect of 2,4-dinitrophenol on K⁺ uptake and Na⁺ loss by low-K⁺ mycelium of *Neocosmospora vasinfecta*

DNP (M)	K ⁺ uptake	Na ⁺ loss	K ⁺ uptake/ Na ⁺ loss
None (control)	48.7*	21.0*	2.3
30 μ M	35.8	20.0	1.8
100 μ M	16.9	15.4	1.1
300 μ M	8.8	8.5	1.0

* Values (averages of duplicates) in μ -equiv./100 mg. dry wt. Incubation for 10 min. in DNP (brought to pH 5.7 with NaOH) followed by 2 hr incubation in KC mm-KCl at 24°.

Fig. 3. Effect of KCl concentration on potassium uptake and sodium loss by *N. vasinfecta*. Uptake for 2 hr at 25°. Bars show range of duplicates.

Fig. 4. Effect of mM-sodium azide on potassium uptake and sodium loss at 24.5°. Controls incubated in mM-NaCl. Bars show range of duplicates.

K^+ level at up to a 100-fold equivalent excess over K^+ in the uptake medium. Net loss of Na^+ under these conditions was, however, drastically decreased. Magnesium, when provided at 100-fold equivalent excess ($MgSO_4$ 50 mM; KCl 1 mM), did not affect the time required for K^+ equilibration but depressed the equilibrium K^+ level. This effect was hyperbolic with Mg^{2+} concentration and amounted to a 20.0% decrease in the equilibrium K^+ level at 100-fold equivalent excess of Mg^{2+} . However, there was no effect on simultaneous Na^+ loss.

Calcium acted similarly to Mg^{2+} but was more potent. At twofold equivalent excess ($CaCl_2$ 1 mM; KCl 1 mM), K^+ uptake was decreased 25% after 2 hr but Na^+ loss was unaffected.

Fig. 5. Effect of various concentrations of $RbCl$ on uptake of both Rb^+ and K^+ from mM- KCl by *N. vasinfesta* at 22.5° . Bars show range of duplicates.

The effects of various concentrations of rubidium on K^+ uptake from mM- KCl are shown in Fig. 5. As usual, the net K^+ uptake was essentially complete in 2 hr although Rb^+ continued to accumulate for at least 4 hr. Depression of the equilibrium K^+ level was hyperbolic with Rb^+ concentration, and amounted to 50% at fivefold equivalent excess of rubidium. Depression of the K^+ level was accompanied by an almost equivalent accumulation of Rb^+ , although this relationship would certainly be different for a 4 hr incubation period. As in the case of Mg^{2+} the net Na^+ loss was unaffected by Rb^+ in the KCl solution.

Loss of ions from mycelium. A smaller loss of Na^+ also occurred when low- K^+ mycelium was transferred to distilled water instead of to KCl solutions; this loss was complete within 1 hr and amounted to 20 to 25% of the initial content. Simultaneously a smaller loss of K^+ sometimes took place, not exceeding 14% of the initial content.

The characteristics of K^+ loss by mycelium previously allowed to reach equilibrium in KCl solutions should indicate how the equilibrium K^+ level is established and maintained.

(a) *K⁺ losses to water only.* As with the untreated low- K^+ mycelium, a small loss of potassium took place, complete within 1 hr, when the mycelium was transferred to

distilled water following equilibration in KCl (Table 3). The actual loss of K⁺ was independent of the volume of washing water over a tenfold range. Also it is clear (lines 2 and 6, Table 3) that, following suspension of the mycelium for 1 hr in one volume of water, no further K⁺ was lost on transfer to a second, identical volume of water. Lines 4 and 6 of Table 5 show that K⁺ loss was not enhanced by mM-NaCl. This was also true for 10 mM-NaCl and for 20 mM-sodium phosphate (at pH 6.5).

Table 3. *Loss of potassium to water at 24° by N. vasinfecta following a period of K⁺ uptake (2-hr uptake from mM-KCl at 24°)*

Wash volume (ml.)	Wash time (hr)	K ⁺ level*	Control (%)
—	—	69.9 ± 3.4	100.0
1000	1	65.2 ± 1.4	93.3
1000	2	64.7 ± 4.1	92.6
2000	2	64.9 ± 3.8	92.8
200	2	63.8 ± 3.9	91.3
1000 + 1000	1 + 1	64.6 ± 0.9	92.4

*K⁺ levels (mean of triplicates) expressed as μ -equiv./100 mg. dry wt, $\pm \sigma$. K⁺ level of samples *before* incubation in KCl, 36.4 μ -equiv./100 mg. dry wt.

Table 4. *Potassium loss as influenced by 2,4-dinitrophenol in N. vasinfecta*

Uptake conditions		Washing conditions		K ⁺ level* μ -equiv./ 100 mg. dry wt.
[KCl]	Additions	Solution	Incubation time (hr)	
—	—	—	—	27.5
mm	—	—	—	67.4
	—	Water	1	62.2
	—		3	57.8
	—	0.3 mM-DNP	1	50.3
	—		3	48.9
	0.3 mM-DNP	—	—	34.1

* K⁺ levels averages of duplicates. K⁺ uptake period, 2 hr at 22.5°. (DNP brought to pH 5.7 with NaOH.)

(b) *K⁺ losses to inhibitor solutions.* A comparison of lines 1, 2 and 7 of Table 4 shows that DNP at a concentration already shown to inhibit K⁺ uptake by over 70% (Fig. 4; Table 2), decreased net K⁺ uptake to 16.5% of the control. The resulting steady K⁺ level was just over half that in control samples. When presented to mycelium immediately following equilibration in KCl, however, the DNP was far less effective in decreasing the steady K⁺ level (cf. Table 4, lines 3 to 6). After 3 hr, the K⁺ level in DNP-treated control samples was approximately 85% of that in water-treated control samples. Similar results were obtained with sodium azide (Table 5). Depending on whether pre-equilibration took place in KCl 0.1 M or mM subsequent transfer to sodium azide depressed the K⁺ level to 85 to 91% of NaCl-treated controls (Table 4, lines 2 to 5). In parallel experiments where sodium azide (mM) was presented simultaneously with KCl, the K⁺ level was depressed to less than 50% of that of the controls, irrespective of incubation period (Fig. 4). Therefore, like DNP, azide was less effective in inducing K⁺ loss than in preventing K⁺ uptake.

(c) *K⁺ losses to RbCl solutions.* Potassium loss to RbCl 10 mM was compared with the effect of the same RbCl level on K⁺ uptake from KCl mM. All samples not post-incubated in RbCl were held in distilled water for the same time. Treatment with RbCl was more effective than any of the preceding treatments in inducing K⁺ losses from the mycelium (Table 6). Nevertheless, the K⁺ level was not decreased to that of mycelium which had absorbed K⁺ in the presence of RbCl, even after twice as long an exposure to Rb⁺ (line 7 and line 5, Table 6). The loss of potassium was insignificant after the second hour, despite continuing uptake of Rb⁺. After 4 hr, the Rb⁺:K⁺ ratio in the post-incubation medium in this experiment exceeded 200, yet the K⁺ level was not decreased to that found for a Rb⁺:K⁺ ratio of 10 in the uptake medium. Therefore, the bulk of mycelial K⁺ was not removed by Rb⁺ ions.

Table 5. *Potassium loss as influenced by sodium azide in N. vasinfector*

Uptake soln.	Washing soln. (mM)	K ⁺ level	Na ⁺ level
—	—	18.1	21.1
0.1 M-KCl	NaCl	58.6	10.2
	NaN ₃	53.3	11.3
10 mM-KCl	NaCl	59.5	6.1
	NaN ₃	50.9	8.0
	KCl	61.2	0.6

Ion levels (averages of duplicates) in μ -equiv./100 mg. dry wt. K⁺ uptake, 2 hr at 23.5°. Washing period, 1 hr at 23.5°.

Table 6. *Effect of RbCl on the equilibrium K⁺ level in N. vasinfector*

Uptake medium	Washing medium (hr)	K ⁺ level* Rb ⁺ level* μ -equiv./100 mg. dry wt	
—	—	10.08	—
10 mM-KCl	Water, 2	51.98	—
	Water, 4	50.40	—
10 mM-KCl + 10 mM-RbCl	Water, 2	20.14	35.44
	Water, 4	20.63	38.75
10 mM-KCl	10 mM-RbCl, 2	36.97	19.38
	10 mM-RbCl, 4	35.13	32.45

* Ion levels (averages of duplicates) K⁺ uptake for 2 hr. Temperature, 23°.

DISCUSSION

The absorption of K⁺ and of other ions by a variety of organisms leads to an equilibrium condition at which no further net uptake occurs. Equilibrium is reached within minutes or hours in bacteria (Schultz, Epstein & Solomon, 1963; Galdiero, 1966), while for roots of higher plants one or more days may be required (Jackson & Edwards, 1966). In the present experiments with *Neocosmospora vasinfector* approximately 2 hr sufficed, for a wide range of external K⁺ concentrations. In general, and notably for monovalent ions, the equilibrium appears to be dynamic, with efflux balancing influx (Rothstein, 1959). Although no data on simultaneous K⁺ fluxes are as yet available for *N. vasinfector*, the present work provides some information about efflux from K⁺-loaded mycelium. Experiments involving washing such mycelium in water or inhibitor

solutions (Tables 3 to 5) indicate that simple diffusive efflux (leakage) is unlikely to play a major role. On the other hand, the effect of Rb⁺ on K⁺ loss (Table 6) is most easily explained as exchange diffusion, in which K⁺ leaves in exchange for Rb⁺ entering the mycelium. The specificity of K⁺ loss towards Rb⁺ and Na⁺ resembles that of K⁺ uptake, suggesting that the same transport system may be involved in both processes. Equilibria mediated by a K⁺ exchange system are known in other micro-organisms (Epstein & Schultz, 1966; Slayman & Tatum, 1965).

Table 6 also shows that a relatively large proportion of mycelial K⁺ appears to be unavailable for exchange with Rb⁺ when the latter is presented after K⁺ uptake is complete. Two explanations suggest themselves, both of which involve a secondary sequestration of K⁺ following its entry into the cell. (1) A fraction of cellular K⁺ may become bound in a non-exchangeable form after its discharge from the potassium transport system. (2) The secondarily sequestered fraction of cell K⁺ may remain exchangeable but be associated with a cytoplasmic system which discriminates more strongly against Rb⁺ than does the potassium transport system itself. A decision between these alternatives cannot be made without recourse to isotopic potassium. Nevertheless, the potassium level in the mycelium does not appear to be regulated solely by the properties of the transport system itself. The penetration of Rb⁺ into *Neurospora crassa* is apparently followed by its binding into a non-exchangeable form (Lester & Hechter, 1958).

Whereas the equilibrium K⁺ level appears to be associated with K⁺ ⇌ K⁺ exchange, the approach to equilibrium involves exchange of K⁺ with other cations. Loss of Na⁺ is usually insufficient to balance K⁺ uptake, and little chloride accompanies K⁺ into the cells, at least during the first hour. It is reasonable to suppose that the excess K⁺ absorbed is balanced by excretion of H⁺, as in *Escherichia coli* (Schultz *et al.* 1963) and in yeast (Conway & O'Malley, 1946). Where measured, the actual pH decrease during K⁺ uptake exceeds any expected pH decrease due to such an exchange, but the pH changes taking place in distilled water only were never observed.

The mechanism mediating K⁺ ⇌ Na⁺ exchange is unknown, but the operation of a classical sodium pump is not supported by either the wide fluctuations in the K⁺:Na⁺ transport ratio or the insensitivity to ouabain.

Lack of information on the trans-surface electrical potential difference prevents a final decision whether K⁺ uptake in *Neocosmospora vasinfecta* is active or not (Ussing, 1949). Values of this potential difference have been reported for *Neurospora crassa* which will account for a 10,000-fold accumulation of K⁺ in cells as compared with external medium, without invoking active transport (Slayman, 1965*a*). However, the effects of sodium azide and 2,4-dinitrophenol on K⁺ uptake (Table 2; Fig. 4) strongly indicate the participation of energy metabolism in this process. These inhibitors do not act merely by rendering the cellular membranes leaky (Tables 4, 5; Slayman & Tatum, 1964). Two other modes of action are possible: (a) that potassium influx depends, at least partly, on energy transfer; or (b) that the trans-surface electrical potential difference is made less negative. If the K⁺ transport system is electrogenic, as Slayman (1965*b*) has concluded for *N. crassa*, then it may be impossible to distinguish between these alternatives. For the present, it is sufficient to conclude that K⁺ uptake is a metabolic process, although it may not qualify as active transport.

A detailed comparison of the K⁺ transport in *Neocosmospora vasinfecta* with that of other fungi is not possible on the basis of the present studies. The use of low-K⁺

mycelium in these experiments is analogous to the production of sodium-yeast (Conway & Moore, 1954). In the importance of ion-exchange processes to net K^+ uptake and maintenance of the equilibrium K^+ level, and in the sensitivity of K^+ uptake to alterations of cellular energy metabolism *N. vasinfecta* shows a general similarity to other fungi (Rothstein, 1959; Slayman & Tatum, 1964, 1965). Discrimination between K^+ and Na^+ by the K^+ -uptake system appears to be greater in *N. vasinfecta* than in yeast (Conway & Duggan, 1958). Measurements of potassium fluxes are a clearly desirable next phase in these studies.

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REFERENCES

- BRUNO, G. A. & CHRISTIAN, J. E. (1961). Determination of carbon-14 in aqueous bicarbonate solutions by liquid scintillation counting technique. *Analyt. Chem.* **33**, 1216.
- BUDD, K. (1969*a*). The assimilation of bicarbonate by *Neocosmospora vasinfecta*. *Can. J. Microbiol.* **15**, 389.
- BUDD, K. (1969*b*). Net transport of potassium by nongrowing fungal mycelium. *XI Intern. Botan. Congr. Proceedings*. (In Press.)
- BUDD, K. & HARLEY, J. L. (1962). The uptake and assimilation of ammonia by *Neocosmospora vasinfecta*. *New Phytol.* **61**, 138.
- CONWAY, E. J. & DUGGAN, F. (1958). A cation carrier in the yeast cell wall. *Biochem. J.* **69**, 265.
- CONWAY, E. J. & O'MALLEY, E. (1946). The nature of the cation changes during yeast fermentation, with formation of 0.02 *N* hydrogen ion. *Biochem. J.* **40**, 59.
- CONWAY, E. J. & MOORE, P. T. (1954). A sodium-yeast and some of its properties. *Biochem. J.* **57**, 523.
- EPSTEIN, W. & SCHULTZ, S. G. (1966). Cation transport in *Escherichia coli*. VI. K exchange. *J. gen. Physiol.* **49**, 469.
- GALDIERO, F. (1966). The kinetics of potassium exchange in cells of *Staphylococcus aureus*. *Biochim. biophys. Acta* **126**, 54.
- HOROWITZ, N. H. & BEADLE, G. W. (1943). A microbiological method for the determination of choline by use of a mutant of *Neurospora*. *J. biol. Chem.* **150**, 325.
- JACKSON, P. C. & EDWARDS, D. G. (1966). Cation effects on chloride fluxes and accumulation levels in barley roots. *J. gen. Physiol.* **50**, 225.
- LESTER, G. & HECHTER, O. (1958). Dissociation of rubidium uptake by *Neurospora crassa* into entry and binding phases. *Proc. natn. Acad. Sci. U.S.A.* **44**, 1141.
- ROTHSTEIN, A. (1959). Role of the cell membrane in the metabolism of inorganic electrolytes by microorganisms. *Bact. Rev.* **23**, 175.
- ROTHSTEIN, A. (1965). *The Fungi: an Advanced Treatise*. Ed. by G. C. Ainsworth and A. S. Sussman, vol. 1, p. 429. New York: Academic Press, Inc.
- SCHULTZ, S. G., EPSTEIN, W. & SOLOMON, A. K. (1963). Cation transport in *Escherichia coli*. IV. Kinetics of net K uptake. *J. gen. Physiol.* **47**, 329.
- SLAYMAN, C. L. (1965*a*). Electrical properties of *Neurospora crassa*. Effects of external cations on the intracellular potential. *J. gen. Physiol.* **49**, 69.
- SLAYMAN, C. L. (1965*b*). Electrical properties of *Neurospora crassa*. Respiration and the intracellular potential. *J. gen. Physiol.* **49**, 93.
- SLAYMAN, C. W. & TATUM, E. L. (1964). Potassium transport in *Neurospora*. I. Intracellular sodium and potassium concentrations and cation requirements for growth. *Biochim. biophys. Acta* **88**, 578.
- SLAYMAN, C. W. & TATUM, E. L. (1965). Potassium transport in *Neurospora*. II. Measurement of steady-state potassium fluxes. *Biochim. biophys. Acta* **102**, 149.
- USSING, H. H. (1949). The distinction by means of tracers between active transport and diffusion. *Acta physiol. scand.* **19**, 43.