Potassium Fluxes in *Neocosmospora vasinfecta*

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SUMMARY

The unidirectional K^+ fluxes across the mycelial surface of *Neocosmospora vasinfecta* were determined using ^{42}K . Influx was mediated by at least two kinetically distinct systems, one having an apparent K_m of $6.5 \mu\text{-equiv. } K^+/l$ and the other of about $1.0 \text{ m-equiv. } K^+/l$. The $V_{\max}$ for both systems was in the range 18 to $22 \mu\text{-equiv. } K^+/100 \text{ mg mycelial dry matter/h}$ (1.0 to $1.2 \text{ m-equiv. } K^+/l$ cell-water/min). Influx was strongly inhibited by 2,4-dinitrophenol, sodium azide, sodium arsenate and anaerobiosis. K^+ efflux was dependent on the external K^+ concentration and ranged from 3 to 10% of mycelial K^+/h . The maximum efflux rate was always considerably less than the initial influx rate for the K^+ concentrations examined. During incubation in dilute KCl solutions, K^+ influx decreased to a value approaching the K^+ efflux rate. It is considered that equilibrium with external K^+ is attained primarily by the regulation of K^+ influx, and that this may be the principal mechanism controlling cytoplasmic K^+ levels.

Adsorption of K^+ was also observed throughout the K^+ concentration range examined and can be attributed to two distinct K^+ -binding entities at the mycelial surface, half-saturating at approximately 0.1 mM- and 4.4 mM-KCl respectively.

INTRODUCTION

Among inorganic ions, potassium appears to have particularly high mobility across the surface membranes of many types of cell. Detailed investigations of K^+ fluxes in fungi have been confined to baker's yeast, *Saccharomyces cerevisiae* (e.g. Rothstein & Bruce, 1958) and *Neurospora crassa* (e.g. Slayman & Tatum, 1965). These studies indicated rather rapid unidirectional fluxes, and this was true in *Neurospora* even when the mycelium was in quasi-equilibrium with respect to the external potassium. In yeast, influx was comparable to that in *Neurospora*, and the approach to equilibrium with external potassium was characterized by increasing efflux, although modulation of influx was also sometimes seen (Rothstein & Bruce, 1958).

In *Neocosmospora* (Budd, 1969*b*), Rb^+ ions had limited ability to remove K^+ ions from the mycelium, although Rb^+ effectively inhibited K^+ uptake. It was considered that K^+/K^+ exchange at the mycelial surface was unlikely to be the only factor involved in regulating the mycelial K^+ level, and it was suggested that much of the mycelial K^+ might not be available for exchange. The present studies with $^{42}K^+$ show that the bulk of mycelial potassium exchanges only slowly with external potassium in *Neocosmospora*, and indicate that regulation of K^+ influx is a key factor in determining net uptake. In addition, some characteristics of potassium adsorption by the mycelium are described.

METHODS

Neocosmospora vasinfecta ATCC11686 was purchased from the American Type Culture Collection and grown in liquid shaken culture at 25 ± 0.5 °C, using Medium G1 (Budd, 1969*a*). The mycelium was harvested while still in exponential growth, when the absorbance (600 nm, 1 cm cell) of a tenfold dilution of the culture was 0.35 to 0.50. The mycelium was separated from growth medium by filtration under reduced pressure, using Whatman No. 54 filter paper circles held in a Millipore filter, and thoroughly washed by resuspension in demineralized water. Replicate samples of 45 to 110 mg dry wt were prepared by taking equal volumes of the well-stirred final suspension, filtering as above, and transferring to experimental solutions. Mycelial suspensions (0.08 to 0.15 mg dry matter/ml) were incubated at 22 to 25 °C and continually agitated by a stream of air, or, where anaerobiosis was required, oxygen-free N₂ gas. For studies of influx or efflux, suspensions were placed in glass columns and periodically sampled via a wide-bore glass stopcock at the bottom of the column. The pH of the unbuffered suspensions was between 5.3 and 5.8, and preliminary experiments showed that pH shifts within this range had a negligible effect on influx rate.

For the determination of unidirectional K⁺ fluxes, ⁴²K was used. This was received from I.C.N., Irvine, California, U.S.A., as K₂CO₃ (approx. 10 mCi/m-equiv. K⁺) or KCl (approx. 25 mCi/m-equiv. K⁺) in aqueous solution, or from the McMaster University Nuclear Reactor, Hamilton, Ontario, Canada, as K₂CO₃ powder (4 to 10 mCi/m-equiv. K⁺). The carbonates were neutralized with analytical grade HCl. The isotope was presented to the mycelium as aqueous KCl (0.1 to 1.2 mCi/m-equiv. K⁺). To estimate ⁴²K⁺ content, samples of mycelium were filtered free of radioactive media as described above and scraped from the filter paper with a stainless steel spatula. They were transferred to plastic vials and counted as soon as possible in a Nuclear Chicago model 4233 automatic gamma counting system. Simultaneously, a portion (10 or 25 µl) of the labelled KCl used in that particular experiment was counted, to correct for radioactive decay and to convert the observed mycelial radioactivity to equivalents of potassium where necessary. The counting error was less than 1 %.

Total potassium in mycelia was estimated by flame emission spectrophotometry using a Unicam SP90A absorption/emission flame spectrophotometer, after digesting the sample with conc. HNO₃.

Demineralized water used throughout had a specific resistance in excess of 1.0 MΩ/cm³. Valinomycin was purchased from Sigma, 2,4-dinitrophenol from I.C.N.-K & K Laboratories, Plainview, New York, U.S.A., and sodium azide from BDH. All other chemicals used were analytical grade.

RESULTS

Time relationships and kinetics

Despite the high K⁺ content of mycelium grown in Medium G1 (Budd, 1969*b*), a small net uptake of K⁺ occurred when the mycelium was transferred to dilute KCl solutions. This uptake was too small in relation to initial K⁺ content for accurate determinations of net K⁺ influx to be made. Preliminary experiments suggested that this net uptake was at least partly a response to the osmotic swelling which occurred when the mycelium was transferred from the growth medium (approx. 0.3 osm at harvest) to these more dilute solutions. Initially, attempts were made to measure the 'steady-state' K⁺ influx in mycelia pre-equilibrated in non-radioactive KCl solutions of various concentrations for 2 to 3 h.

Fig. 1

Fig. 2

Fig. 1. The progress of influx of K^+ from $25 \mu M$ - ^{42}KCl by mycelium pre-incubated either in water (continuous lines) or in non-radioactive $25 \mu M$ - KCl (broken lines). Pre-incubation periods were: ○, 2 min; ●, 22 min; □, 64 min; ■, 86 min; △, 150 min, ▲, 162 min. Temperature, $23^\circ C$.

Fig. 2. Double reciprocal plot for rates of K^+ influx for the concentration range 2.0 to $50.0 \mu M$ - KCl . Temperature, $23^\circ C$.

The influxes measured in this way were not reproducible and were very low compared with the maximum K^+/Na^+ exchanges rates already observed (Budd, 1969*b*). Rather than extend the pre-equilibration period, the K^+ influx rates of mycelium freshly harvested and washed were examined as a function of time of pre-incubation in demineralized water. Under these conditions, K^+ influxes proved to be quite reproducible (Fig. 1), at least for the first 80 to 90 min incubation in demineralized water. In contrast, mycelium pre-incubated in $25 \mu M$ - KCl solution showed a progressive decline in influx rate with increased time of pre-incubation. However, influx appeared always to be linear with time for approximately 12 min following an initial rapid adjustment (adsorption, see below). Every estimate of influx rate represents the slope of an influx time-course based on at least four consecutive determinations of radioactivity taken within an 8 to 12 min exposure to $^{42}K^+$. The contribution of adsorption is thus not included in influx determinations, and is discussed separately below.

The kinetics of K^+ influx were examined using mycelium held in water for a maximum of 1 h before exposure to $^{42}K^+$. Figure 2 shows the results for the range 2 to $50 \mu M$ - KCl . These indicate a single kinetic system mediating K^+ influx in this concentration range, with an apparent K_m (data of Fig. 2) of 6.7μ -equiv. K^+/l and a V_{max} of approximately 18.0μ -equiv. $K^+/100$ mg dry wt/h (average values from three estimates; K_m 6.3μ -equiv. K^+/l , V_{max} 17.4μ -equiv. $K^+/100$ mg dry wt/h). *Neocosmospora* thus possesses a K^+ transport system of relatively high affinity for K^+ .

The specificity of this transport system also appeared to be high. In the presence of

Fig. 3. Double reciprocal plots for rates of K^+ influx at 24°C for the concentration ranges (a) 0.025 to 15.0 mM-KCl, and (b) 0.15 to 15.0 mM-KCl. The data for (b) are those for (a) corrected for the contribution of the high-affinity K^+ influx system (see text).

either Na^+ or Mg^{++} at 2.5 m-equiv./l, K^+ influx from 25 μM -KCl was depressed by only approximately 40 %.

Influx kinetics in the range 25 μM - to 15 mM-KCl are presented in Fig. 3(a) and (b). The double reciprocal plot in Fig. 3(a) is strongly curved, indicating the participation of more than a single system for K^+ influx over this concentration range. Extrapolation of the tangent to the upper arm of this curve gives an intercept on the abscissa corresponding to a K_m of 7.0 μM , and its ordinate intercept yields a value for $V_{\max}$ of 18.5 $\mu\text{-equiv. K}^+ / 100 \text{ mg dry wt/h}$. Both values clearly refer to the high-affinity system already discussed. All other influx values in Fig. 3(a) must be corrected for influx via this system, and it appears that the kinetic characteristics of the influx system are sufficiently distinct for this to be a simple operation. Using the Briggs-Haldane equation:

$$v_1 = \frac{V_{\max}[S]}{K_m + [S]},$$

where v_1 indicates influx via the high-affinity system only, $[S]$ the KCl concentration in mM, and $V_{\max}$ and K_m have the values given above, v_1 is calculated for all KCl concentrations in Fig. 3(a) and subtracted from the observed influx. The resulting values are replotted in Fig. 3(b), and form a moderately good fit to the line shown. The values of K_m and $V_{\max}$ given by this line are, respectively, 1.0 mM and 21.7 $\mu\text{-equiv. K}^+ / 100 \text{ mg dry wt/h}$. *Neocosmospora* thus appears to possess both a low- and a high-affinity system for K^+ influx.

The properties of this low-affinity system have not been extensively examined because of the technical difficulty of separating it from the high-affinity system in normal mycelium. Further experiments were confined to examining this latter system, except as noted below.

Metabolic aspects of K^+ influx

Table 1 shows the effects of several metabolic inhibitors and anaerobiosis on K^+ influx. Dinitrophenol and sodium azide were added to the mycelial suspension immediately before the ^{42}KCl : the dinitrophenol was at a concentration known to uncouple respiration

Table 1. *The effects of metabolic inhibition on K⁺ influx and adsorption*

Influx was measured over 12 min after addition of ⁴²KCl. Adsorption was determined as the ordinate (zero-time) intercept extrapolated from each influx time-course. 2,4-Dinitrophenol was adjusted to pH 5.6 with NaOH, and sodium azide and sodium arsenate to pH 5.8 with H₂SO₄. The pH of control suspensions was 5.4 to 5.5.

Expt no.	Treatment	Influx (μ -equiv. K ⁺ / 100 mg dry wt/h)	Adsorption (μ -equiv. K ⁺ / 100 mg dry wt)
1	Control (25 μ M-KCl only)	11.68	0.88
	10 ⁻⁴ M-2,4-dinitrophenol	0.20	0.25
	0.09 mM-NaCl*	10.96	0.94
	10 ⁻³ M-sodium azide	0.04	0.20
	10 ⁻³ M-sodium chloride†	8.00	0.55
2	Control (25 μ M-KCl)	11.50	0.80
	Anaerobic	0.15	0.93
3	Control (25 μ M-KCl)	16.70	0.50
	Sodium arsenate (1.0 mM)	0.40	0.45
	Sodium chloride (2.0 mM)‡	10.40	0.45
4	Control (5 mM-KCl)	44.80	12.00
	10 ⁻⁴ M-2,4-dinitrophenol	4.40	11.55
	Anaerobic	0.80	12.55

* Control for 2,4-dinitrophenol.

† Control for sodium azide.

‡ Control for sodium arsenate.

in *Neocosmospora* (Miller & Budd, 1975). Both of these inhibitors gave very marked (90 to 99 %) inhibition of K⁺ influx within 3 min. Anaerobiosis was attained by suspending the mycelium in deoxygenated water and bubbling with N₂ gas for 5 min before the addition of ⁴²KCl. At both 25 μ M- and 5 mM-KCl, anaerobiosis gave over 98 % inhibition of K⁺ influx. The short deprivation of oxygen in these experiments would not lead to irreversible physiological effects (Miller & Budd, 1975). Arsenate (experiment 3) was tested both with and without a 5 min pre-incubation before adding ⁴²KCl, but only the results for mycelium pre-incubated in the inhibitor are presented. Influx was unaffected by arsenate at this concentration for 4 to 6 min, after which there was essentially complete inhibition.

Table 1 also shows the effects of metabolic inhibition on K⁺ adsorption (determined by the graphical method, see below). As expected, neither anaerobiosis nor sodium arsenate reduced adsorption when compared with the appropriate control. At 25 μ M-KCl, however, both dinitrophenol and sodium azide strongly depressed adsorption. At 5.0 mM-KCl, dinitrophenol scarcely affected adsorption.

Glucose (1.0 mM) slightly stimulated influx from 25 μ M-KCl (up to 30 %); its effect at 5.0 mM-KCl was not tested. Net potassium uptake was not increased by glucose (see also Budd, 1969*b*).

Valinomycin at 1.0 to 4.0 μ g/ml had no measurable influence on influx from 25 μ M-⁴²KCl.

Efflux of K⁺

Potassium efflux was examined after only short periods (1.0 or 2.5 h) of loading with ⁴²K⁺: no attempt was made to grow *Neocosmospora* in the presence of the isotope. Figure 4 shows efflux into 25 μ M-KCl following 1 or 2.5 h preloading with ⁴²K⁺ from 25 μ M-KCl. Following an initial adjustment (desorption?) which appeared to be complete

Fig. 4

Fig. 4. The efflux of $^{42}\text{K}^+$ to $25\ \mu\text{M}$ non-radioactive KCl. Mycelium was preloaded in $25\ \mu\text{M}$ - ^{42}KCl for (O) 1 h or (●) 2.5 h. Temperature, 24°C . The results of two parallel experiments are shown.

Fig. 5

Fig. 5. Scatchard plot of the data for K^+ adsorption for the concentration range 0.025 to $15.0\ \text{mM}$ -KCl. O, Original data; ●, replotted data (see text). All curves fitted by inspection. Temperature, 24°C .

within 5 min (the shortest efflux period measured), efflux of $^{42}\text{K}^+$ was extremely slow. The exact pattern of efflux could not be discerned from these data, because the sampling error was appreciable compared with the actual loss. For simplicity, however, the post-desorption efflux in Fig. 4 is represented as taking place from a single kinetic compartment. On the assumption that all mycelial K^+ (other than that adsorbed to the mycelial surface) was located within this compartment, a maximum value for K^+ efflux was calculated by multiplying the percentage loss per hour from the compartment, by the total K^+ within it. Table 2 presents estimates of efflux made in this way, with measurements of influx from parallel experiments where these were available. Whereas influx decreased markedly after 150 min in $25\ \mu\text{M}$ -KCl as compared with 60 min, efflux to $25\ \mu\text{M}$ -KCl was not significantly changed. The estimated half-times for $^{42}\text{K}^+$ loss from the mycelium were 20.3 h for 60 min preloading, and 18.2 h for 150 min. Influx considerably exceeded efflux after 60 min in $25\ \mu\text{M}$ -KCl, but was about equal to it after 150 min. These results show that the mycelium was close to equilibrium with $25\ \mu\text{-equiv. K}^+/\text{l}$ after 150 min, and indicate that equilibrium was approached primarily by regulation of K^+ influx.

The data concerning 5 mM-external KCl (at which concentration both influx systems are operating) are less extensive but basically confirm those above. Efflux was about three times as fast to 5 mM-KCl as to $25\ \mu\text{M}$ (Table 2) and the exchange half-time was approximately 6.7 h. Influx rate was close to that of efflux after 1 h in 5 mM-KCl, and was about $7.0\ \mu\text{-equiv.}/100\ \text{mg dry wt/h}$. This, however, was far below the initial influx rate observed in 5 mM-KCl, which was approximately $45\ \mu\text{-equiv.}/100\ \text{mg dry wt/h}$ (Table 1). Hence, equilibrium was again attained mainly by reduction of influx.

Under anaerobiosis, or with $10^{-4}\ \text{M}$ -dinitrophenol, efflux rate was approximately doubled (Table 2). The half-times for $^{42}\text{K}^+$ loss under these conditions were therefore decreased

Table 2. *Efflux and influx of K⁺ under various conditions*

Mycelium was preloaded for the times shown, using ⁴²KCl for efflux studies and non-radioactive KCl for influx. It was then transferred to KCl of the same concentration for flux measurement (non-radioactive for efflux studies, ⁴²K⁺-labelled for influx). The average values of α (the specific loss factor) for radioactive K⁺ were as follows: to 25 μ M-KCl, 0.034/h for 60 min preloading and 0.038/h for 150 min preloading; to 5 mM-KCl, 0.103 (60 min preloading only).

Efflux conditions	KCl concn. during preloading	Pre-loading period (min)	Efflux (μ -equiv. K ⁺ /100 mg dry wt/h)			Influx (μ -equiv. K ⁺ /100 mg dry wt/h)		
			Mean	S.E.M.	N	Mean	S.E.M.	N
25 μ M-KCl	25 μ M	60	2.41	0.39	12	9.45	0.11	4
25 μ M-KCl	25 μ M	150	2.47	0.78	6	3.00	0.25	4
25 μ M-KCl + 0.1 mM-DNP	25 μ M	60	5.07	—	2	—	—	—
25 μ M-KCl, anaerobic	25 μ M	60	4.93	—	2	—	—	—
5 mM-KCl	5 mM	60	7.74	—	2	7.0*	—	—

N, no. of observations.

* One sample only.

but still were of the order of 8 to 10 h, even though the energy supply to the influx systems was presumably almost abolished (see Table 1). These results indicate that potassium was not retained within the mycelium primarily by mechanisms dependent on the expenditure of respiratory energy. The mycelial surface membrane was evidently only slightly permeable to K⁺ ions in the outward direction.

Valinomycin, at 4 μ g/ml, did not increase ⁴²K⁺ efflux to 25 μ M-KCl during a 2 h exposure. The efflux of ⁴²K⁺ to water only was reduced as compared with KCl solutions (specific loss factor, α = 0.005 to 0.019/h).

Adsorption of K⁺

The influx time-courses, though linear with time after the earliest sampling, did not extrapolate to pass through the origin at zero time (cf. Fig. 1). Extrapolation gave a positive intercept on the uptake axis, and this was taken to represent K⁺ adsorption. Adsorbed ⁴²K⁺ was not completely removed by brief desorption into non-radioactive KCl. Resuspension of the mycelium for up to 30 s in 10 mM-KCl removed 95% of the ⁴²K⁺ adsorbed from 5 mM-⁴²K⁺, but only approximately 65% of that adsorbed from 50 μ M-⁴²KCl. Potassium adsorption was thus a heterogeneous phenomenon.

Adsorption of K⁺ was appreciable even in the micromolar concentration range. Figure 5 shows the relationship between K⁺ adsorbed and external K⁺ concentration, plotted according to the method of Scatchard (1949). The curve drawn is the graphical representation of the equation

$$\bar{v}/c = K(n - \bar{v})$$

where $\bar{v}$ is the average value of K⁺ adsorbed per unit dry weight ('bound' K⁺), c is the concentration of K⁺ ions in external solution ('free' K⁺), n the maximum possible K⁺ bound, and K the association constant. When $\bar{v}/c$ is plotted against $\bar{v}$, a straight line results if K is constant over the concentration range employed. The intercept on the $\bar{v}/c$ axis is equal to Kn , and that on the $\bar{v}$ axis equals n .

The KCl concentrations used in this experiment ranged from 25 μ M to 15 mM, and it is clear that the data did not fit a single straight line. The curvature of the line suggested that more than one chemical species (or 'site') at the mycelial surface was responsible for the adsorption of K⁺. Using this approach (see Scatchard, Coleman & Shen, 1957), it was possible to resolve the curve of Fig. 5 into two approximations to straight lines.

Table 3. *Characteristics of adsorption sites for K⁺*

Site	Association constant, <i>K</i> (l/mol)			Affinity, 1/ <i>K</i> * (mol/l)	Abundance, <i>n</i> (μ-equiv./100 mg dry wt)			Relative abundance (%)
	Mean	S.E.M.	<i>N</i>		Mean	S.E.M.	<i>N</i>	
1	9.67×10^3	2.24×10^3	3	1.03×10^{-4}	3.6	0.7	3	16.0
2	2.25×10^2	0.44×10^2	4	4.4×10^{-3}	17.7	3.8	4	84.0

* K⁺ concentration for half-saturation.

Assuming that the binding of K⁺ at the lowest concentrations of KCl described the contribution of a single binding species, the asymptotic tangent was drawn to this (left-hand) arm of the curve (see Fig. 5). From the intercepts of this tangent on the axes, the values of *K*₁ and *n*₁ (subscripts designate 'site 1') were obtained as already described. Values from Fig. 5 were: *K*₁ = 6.5×10^3 l/mol; *n*₁ = 4.8 μ-equiv. K⁺/100 mg dry wt.

The tangent itself was then used to correct all other experimental points for the contribution ($\bar{v}_1$) of site 1 at all other concentrations of KCl. Values of $(\bar{v} - \bar{v}_1)/c$ were then replotted against $(\bar{v} - \bar{v}_1)$, and the resulting points in Fig. 5 fitted a single straight line. Values of *K*₂ and *n*₂ obtained from this line were: *K*₂ = 1.4×10^2 l/mol; *n*₂ = 23.3 μ equiv. K⁺/100 mg dry wt. Hence, only two binding species for K⁺ need be postulated for the concentration range described. Table 3 summarizes available experimental data on these binding species for *Neocosmospora*. Site 1 was half-saturated at 0.1 mM-KCl and accounted for about one K⁺ ion-binding site in six. Site 2 was half-saturated at approximately 4.4 mM-KCl and accounted for the remaining K⁺-binding sites.

Data already presented indicated that the two binding sites differ in reversibility of K⁺ binding and in their response to certain inhibitors. The anomalous results obtained with desorption into unlabelled KCl (see above) suggested that adsorption of K⁺ to site 2 (which predominates at 5 mM-KCl) was easily and completely reversible, whereas adsorption to site 1 (which predominates at 25 μM-KCl) was not. Similarly, the contrasting effects of dinitrophenol on adsorption at 5 mM- and at 25 μM-KCl (Table 1) indicated that only site 1 was sensitive to this inhibitor.

DISCUSSION

A striking feature of the above results was the low magnitude of the potassium fluxes compared with the maximum values in the literature for other fungi. In both *Neurospora* and baker's yeast, a single K⁺ influx system has been reported, with an apparent *K_m* for potassium in the millimolar concentration range and *V_{max}* in the region of 20 m-equiv K⁺/l cell-water/min (Slayman & Tatum, 1965; Armstrong & Rothstein, 1965). The fluxes expressed in this paper as μ-equiv. K⁺/100 mg dry wt/h can be converted to the above units by dividing by 18 (in *Neocosmospora*, 100 mg dry matter is equivalent to 0.30 ml cell-water; Miller & Budd, 1975). Hence *V_{max}* for either the high- or the low-affinity K⁺ influx system in *Neocosmospora* was approximately 1 m-equiv. K⁺/l cell-water/min, or roughly 5% of the values for *Neurospora* or yeast. However, because *Neocosmospora* differs from these other species in also possessing a high-affinity transport-system for K⁺, potassium influx from K⁺ concentrations below 50 μ-equiv./l should be more rapid in *Neocosmospora* than in these other fungi. Despite these differences, K⁺ influx was closely linked to metabolism in all three fungi.

Potassium efflux was much less rapid in *Neocosmospora* than in *Neurospora*. The half-time for K⁺ exchange in *Neurospora* mycelium in equilibrium with 5 mM-KCl was

of the order of 6 to 7 min (Slayman & Tatum, 1965). In *Neocosmospora*, it was approximately 6 to 7 h. K^+ efflux in *Neurospora* appeared to be strictly an exchange flux, but in *Neocosmospora* the evidence on this point was inconclusive. Efflux clearly increased as the external K^+ concentration (and rate of influx) increased, as would be expected of an exchange flux: however, anaerobiosis or dinitrophenol, which essentially eliminate influx, actually caused efflux to increase. Under these conditions, a strict exchange flux should be abolished. It is possible that K^+ efflux in *Neocosmospora* represented a slow diffusion leak, reduced still further by recapture. Potassium efflux in baker's yeast, which appeared to be a diffusive leak, was of the same order as in *Neocosmospora* (0.5 m-equiv. K^+ /l cell-water/min or less; Rothstein & Bruce, 1958).

The low K^+ fluxes observed in *Neocosmospora* appeared to reflect both a low passive permeability of the mycelial surface membrane to K^+ , and strict metabolic control over the energy-dependent influx processes. The approach to equilibrium with external K^+ solutions (and hence the regulation of mycelial K^+ content) appeared to involve a decrease in K^+ influx, rather than an increase in K^+ efflux. One advantage of such a method of regulation would be the conservation of respiratory resources. In baker's yeast, equilibration with external K^+ solutions involved a time-dependent increase in K^+ efflux, though at external K^+ concentrations above 1 m-equiv. K^+ /l there was evidence that influx was reduced after prolonged uptake (Rothstein & Bruce, 1958). Whether *Neurospora* is similar is not clear; however, the exchange fluxes of K^+ at equilibrium were much greater than in *Neocosmospora* (see above).

The internal signals responsible for regulating K^+ influx in *Neocosmospora* have not been identified. It is probable that cytoplasmic K^+ concentration is one regulatory factor, but if net K^+ uptake involves exchange for H^+ ions (Budd, 1969*b*) then cytoplasmic pH might be another. Uptake of K^+ in exchange for Na^+ in low-potassium mycelium of *Neocosmospora* (Budd, 1969*b*) was up to fourfold faster than the maximum K^+ influx from the same KCl concentration with normal mycelium, as observed in the present work. This suggests that mycelial K^+ content did influence K^+ influx rate, but since the low- K^+ mycelium was grown on limited K^+ , further work will be necessary to separate direct (inhibitory) effects of mycelial K^+ from possible indirect (repressive) effects on influx.

The data on adsorption in this paper are essentially preliminary, and no information is available concerning the chemical nature or cytological location of the adsorption sites. Adsorption is commonly observed during cation uptake in fungi (Rothstein & Hayes, 1956; Budd & Harley, 1962; Ponta & Broda, 1970; Shere & Jacobson, 1970; Paton & Budd, 1972), as in other organisms, but it is less conspicuous with the monovalent cations than with di- or polyvalent ones. Adsorption of K^+ was observed in yeast (Rothstein & Hayes, 1956), where it displaced Mn^{2+} from one of the two binding sites for this ion. The association constant calculated from their data is approximately 2×10^3 l/mol, or very close to that for site 2 in *Neocosmospora*. However, the total surface binding of cations by yeast was no more than 3 mmol/kg cells (Rothstein & Hayes, 1956) whereas in *Neocosmospora* this figure is at least 50 mmol/kg cells. With *Neurospora*, potassium adsorption was not appreciable below pH 5.8 (Slayman & Slayman, 1970), and at pH 8 reached a maximum value of approximately 25 μ -equiv./100 mg dry wt, similar to that observed for *Neocosmospora* at pH values between 5.5 and 6.0. The association constant for K^+ , however, was very low (31 l/mol), and only a single adsorption site was discernible. Evidently, these three fungi differ considerably in their surface properties, as well as in the characteristics of their K^+ fluxes.

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