BBA 75482

# NET POTASSIUM TRANSPORT IN NEUROSPORA: PROPERTIES OF A TRANSPORT MUTANT

CAROLYN W. SLAYMAN

*Departments of Microbiology and Physiology, Yale University School of Medicine, New Haven, Conn. 06510 (U.S.A.)*

(Received March 19th, 1970)

SUMMARY

Net $K^+$ uptake by wild-type Neurospora shows a complex dependence on the extracellular pH. From pH 4 to 6, uptake follows standard Michaelis–Menten kinetics as a function of external potassium but shifts to sigmoid kinetics at pH 8. Net flux measurements have now been carried out on mutant strain R2449, isolated by virtue of an abnormally high potassium requirement for growth, and previously shown to have an elevated $K_{1/2}$ for steady-state $K^+$ exchange at pH 5.8. The results indicate that R2449 is abnormal in net $K^+$ uptake at pH 4, 5.8 and 8, consistent with the notion that a single cation transport system exists in Neurospora, capable of functioning over the entire pH range. Two models are presented, an allosteric model and a 2-site model, which can account quantitatively for the shift from Michaelis–Menten kinetics at low pH to sigmoid kinetics at high pH and for the defect in R2449. Further experiments will be needed to distinguish between the models.

## INTRODUCTION

Like most other cells, the fungus *Neurospora crassa* maintains a high intracellular potassium concentration, 180–200 mmoles/kg cell water, during logarithmic growth in medium with as little as 0.3 mM $K^+$ (ref. 1). In an attempt to characterize the transport system (or systems) responsible for regulating the $K^+$ content of Neurospora, we have previously measured potassium fluxes under three sets of conditions. (a) Normal high-$K^+$ cells, harvested and resuspended in buffer at pH 5.8 (the pH of the growth medium), carry out a steady-state exchange of internal $K^+$ for external $K^+$, measurable with the isotope $^{42}K$ (ref. 2). (b) Cells depleted of $K^+$ and loaded with $Na^+$ by growth in limiting-$K^+$ medium, tested under similar conditions (pH 4–6), show a net uptake of $K^+$ which is balanced by the efflux of $Na^+$ and $H^+$ (ref. 3). Both of these processes, $K^+/K^+$ and $K^+/Na^+,H^+$ exchange, are energy dependent and follow standard Michaelis saturation kinetics as a function of the external $K^+$ concentration. (c) Above pH 7, by contrast, net $K^+$ uptake follows a sigmoid curve as a function of the external $K^+$ concentration, indicative of multisite transport under these conditions[4]. ($K^+/K^+$ exchange has not been measured at high pH.)

Abbreviations: DMG, 3,3-dimethylglutaric acid; HEPES, *N*-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

The relationship among these three modes of $K^+$ uptake is not yet clear. Perhaps the simplest possibility is that they are all mediated by the same system which, at low pH, shows standard Michaelis (one-site) kinetics for $K^+$ uptake and can transport $Na^+$, $H^+$ or $K^+$ out of the cell, depending upon the affinities of the system for the three cations and upon their relative intracellular concentrations. At high pH, the shift to sigmoid kinetics could be viewed in at least two ways under the single-pump hypothesis: either (i) the transport system contains a second site that must be filled with a cation ($H^+$ at low pH, $K^+$ at high pH) in order for transport to occur, or (ii) the transport system is an allosteric protein consisting of multiple subunits, each with a binding site for $K^+$; cooperative interactions among the subunits give rise to a sigmoid curve at high pH, but $H^+$ ions serve as allosteric activators of the system, causing a shift toward a standard Michaelis curve at low pH.

Alternatively, there might be two distinct cation transport systems in the Neurospora membrane, a conventional one-site system with a low pH optimum and a multisite system with a high pH optimum. Several other instances exist where a single substrate is thought to be transported by two or more independent routes: for example, inorganic phosphate in *Streptococcus faecalis*[5,6], methionine in *Penicillium chrysogenum*[7], arginine, lysine and methionine in *Saccharomyces cerevisiae*[8–10], and most of the neutral amino acids in Neurospora[11].

The present experiments were undertaken as a direct test of the relationship between $K^+/K^+$ exchange, net $K^+$ uptake at low pH, and net $K^+$ uptake at high pH, by examining the properties of a $K^+$ transport mutant of Neurospora. Strain R2449, which maps near leu-1 on linkage group III, was isolated by virtue of its abnormally high $K^+$ requirement for growth and has already been shown to have an elevated $K_{½}$ for $K^+/K^+$ exchange at pH 5.8 (ref. 12). Corresponding shifts in the velocity *vs.* concentration curves for net $K^+$ uptake—at low pH, high pH or both—would suggest that these fluxes are mediated by the same system.

## METHODS

### *Preparation of low-$K^+$ cells*

Wild-type strain RL21a and mutant strain R2449-1-74A of *N. crassa* were used throughout these experiments. To prepare $K^+$-depleted cells for flux measurements, conidia were inoculated at a density of $1 \cdot 10^6$/ml into medium initially containing 0.2 mM $K^+$ (ref. 3) and incubated on a reciprocating shaker at 25° for 16 h (RL21a) or 24 h (R2449). Table I compares the intracellular $K^+$ and $Na^+$ concentrations for cells grown in the 0.2 mM $K^+$ medium with those for cells grown in the normal high-$K^+$ medium. Previous experiments have shown that $K^+$ depletion does not affect the rate of $K^+$ uptake in Neurospora; $v_{max}$'s of 20 and 22.7 mmoles/kg cell water·min have been obtained with normal and depleted cells, respectively, at pH 5.8 (refs. 2, 3).

### *Flux experiments*

Methods for measuring the net fluxes of $K^+$, $Na^+$ and $H^+$ in Neurospora have already been described[3]. Experiments at pH 4 and pH 5.8 were carried out in 20 mM 3,3-dimethylglutaric acid buffer (DMG; Eastman Organic Chemicals); and at pH 8, in 30 mM *N*-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; Calbiochem). All buffers contained 25 mM $Na^+$ and 1 % glucose. Control experiments had shown

Biochim. Biophys. Acta, 211 (1970) 502–512

TABLE I

CATION CONCENTRATIONS IN RL21a (WILD TYPE) AND R2449 (TRANSPORT MUTANT)

All values are mean ± S.E. for 9–33 cell samples.

| | *RL21a* | | *R2449* | |
|---|---|---|---|---|
| | $K^+$ (*mmoles/kg cell water*) | $Na^+$ | $K^+$ (*mmoles/kg cell water*) | $Na^+$ |
| Normal cells, freshly harvested from 37 $K^+$ medium | 180 ± 3 | 14 ± 1 | 152 ± 10 | 19 ± 4 |
| Low-$K^+$ cells, freshly harvested from 0.2 mM $K^+$ medium | 56 ± 1 | 107 ± 4 | 64 ± 2 | 58 ± 2 |
| Low-$K^+$ cells, preincubated 20 min in $K^+$-free buffer (pH 4) | 36 ± 1 | 164 ± 4 | 51 ± 3 | 124 ± 7 |
| Low-$K^+$ cells, preincubated then incubated 40 min in 30 mM $K^+$ (pH 4) | 203 ± 11 | 11 ± 1 | 170 ± 9 | 13 ± 2 |
| Low-$K^+$ cells, preincubated 20 min in $K^+$-free buffer (pH 5.8) | 37 ± 2 | 152 ± 5 | 57 ± 2 | 131 ± 5 |
| Low-$K^+$ cells, preincubated then incubated 40 min in 30 mM $K^+$ (pH 5.8) | 181 ± 5 | 28 ± 3 | 166 ± 5 | 19 ± 2 |
| Low-$K^+$ cells, preincubated 20 min in $K^+$-free buffer (pH 8) | 15 ± 2 | 200 ± 12 | 24 ± 2 | 173 ± 4 |
| Low-$K^+$ cells, preincubated then incubated 40 min in 30 mM $K^+$ (pH 8) | 161 ± 9 | 109 ± 10 | 130 ± 6 | 97 ± 5 |

TABLE II

APPARENT MICHAELIS CONSTANTS AND MAXIMAL VELOCITIES FOR NET CATION FLUXES

At pH 4.0 and 5.8, values of $K_{1/2}$ and $v_{max}$ (± 1 S.E.) were obtained from computer fits of the data in Figs. 1 and 2 to the Michaelis–Menten equation, $v = v_{max}[S]/(K_{1/2} + [S])$, using the MARQUARDT[13] algorithm. This is a generalized program for nonlinear least-squares curve fitting, available under IBM SHARE Distribution No. 3094, and run on the Yale Computer Center IBM 7094/7040 system. At pH 8.0, the data were fitted to a two-site rate equation, $v = v_{max}[S]^2/([K_{1/2}]^2 + [S]^2)$; this equation provides a reasonable description of the behavior of wild-type Neurospora at pH 8 (ref. 4) but is only an approximation for the mutant (see Table IV).

| *Strains* | | *pH 4.0* | | | *pH 5.8* | | | *pH 8.0* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $K_{1/2}$ (*mM*) | $v_{max}$ (*mmoles/kg cell water·min*) | $\sigma^2$ (*variance*) | $K_{1/2}$ (*mM*) | $v_{max}$ (*mmoles/kg cell water·min*) | $\sigma^2$ (*variance*) | $K_{1/2}$ (*mM*) | $v_{max}$ (*mmoles/kg cell water·min*) | $\sigma^2$ (*variance*) |
| RL21a | $K^+$ influx | 11.1 ± 1.1 | 23.4 ± 0.8 | 0.112 | 11.9 ± 1.5 | 23.3 ± 1.0 | 0.298 | 17.8 ± 0.4 | 13.9 ± 0.2 | 0.050 |
| | $Na^+$ efflux | 11.9 ± 2.1 | −17.9 ± 1.3 | 0.140 | 11.6 ± 0.6 | −14.5 ± 0.3 | 0.022 | — | — | — |
| | $H^+$ efflux | — | − 5.2 ± 0.2 | — | 12.3 ± 1.6 | − 8.5 ± 1.1 | 0.176 | — | — | — |
| R2449 | $K^+$ influx | 15.3 ± 1.3 | 23.1 ± 0.8 | 0.075 | 22.3 ± 3.0 | 25.7 ± 1.6 | 0.144 | 24.7 ± 0.9 | 12.6 ± 0.6 | 0.108 |
| | $Na^+$ efflux | 15.6 ± 0.8 | −20.2 ± 1.5 | 0.072 | 26.6 ± 1.1 | −21.9 ± 2.7 | 0.031 | — | — | — |
| | $H^+$ efflux | — | — | — | 22.8 ± 2.3 | − 5.8 ± 1.0 | 0.298 | — | — | — |

previously that these buffers neither support growth nor inhibit growth of wild-type Neurospora and that the passive leak of $Na^+$ and $K^+$ from the cells into the buffers is negligible, averaging only 0.5 mmole/kg cell water·min (ref. 3). Both kinds of control experiments were repeated with R2449, with similar results.

*Calculation of initial rates of $K^+$ uptake and $Na^+$ loss*

At pH 4 and pH 5.8, net $K^+$ uptake and net $Na^+$ loss in R2449, as in RL21a, were simple exponential functions of time, and initial rates were calculated from semilog plots of intracellular concentrations ($[K^+]_\infty - [K^+]_t$ or $[Na^+]_t - [Na^+]_\infty$) *vs.* time (method described fully in ref. 3). At pH 8, cation movements in both strains were more complex; the semilog plots for $K^+$ and $Na^+$ could be resolved into two exponential functions with clearly distinct time constants. The fast component of $K^+$ uptake (with a time constant of 1.1 $\pm$ 0.2 min in R2449 and 1.2 $\pm$ 0.1 min in RL21a) equals the fast component of $Na^+$ loss and is believed to represent ion exchange within the cell wall since it is comparatively insensitive to low temperature and metabolic inhibitors[4]. The slower component (time constant = 10–12 min in both strains at saturating $K^+$ concentrations) is inhibited at 0° and by $CN^-$ and deoxycorticosterone and appears to represent transport across the cell membrane[4]. Initial rates of the transport component were calculated for both strains by carrying out parallel flux experiments in the presence and absence of 1 mM deoxycorticosterone, then subtracting each deoxycorticosterone curve from the corresponding uninhibited curve, making a semilog plot of the difference, and computing the initial rate as at low pH (for a complete description of this method, see ref. 4).

## RESULTS

*Net $K^+$ and $Na^+$ transport at pH 4, 5.8 and 8*

Figs. 1–3 illustrate, for strain R2449, the dependence of net $K^+$ influx and net $Na^+$ efflux on the external $K^+$ concentration at three different pH's. In general the mutant strain follows the same pattern as the wild type, with Michaelis-type saturation curves (and linear double-reciprocal plots) at the low pH's, changing to a sigmoid curve (and a parabola in the double-reciprocal plot) at pH 8. But at all pH's the curves of flux *vs.* concentration are shifted toward higher $K^+$ concentrations in the mutant, slightly at pH 4, progressively more at pH 5.8 and 8.

Table II shows the results of computer fits of these curves. At pH 4 and 5.8, as with wild-type Neurospora[3], the data from R2449 have been analyzed in terms of the Michaelis–Menten equation; at pH 8, again for comparison with the wild type[4], a two-site uptake model has been used. The main results can be summarized as follows:

(1) *$K^+$ influx.* At every pH, the maximal velocity for $K^+$ uptake is essentially the same in the mutant as in the wild type but the $K_{1/2}$ is raised: from 11.1 to 15.3 mM at pH 4, from 11.9 to 22.3 mM at pH 5.8, and from 17.8 to 24.7 mM at pH 8 (these last values were obtained from the two-site rate equation, as described in the legend to Table II).

(2) *$Na^+$ and $H^+$ efflux at low pH.* Previous results on wild-type Neurospora led to the idea that, between pH 4 and 5.8, $K^+$ uptake is coupled to the extrusion of both $Na^+$ and $H^+$ (ref. 3). The major piece of evidence supporting this notion was that the fluxes of all three cations showed the same dependence on the external $K^+$

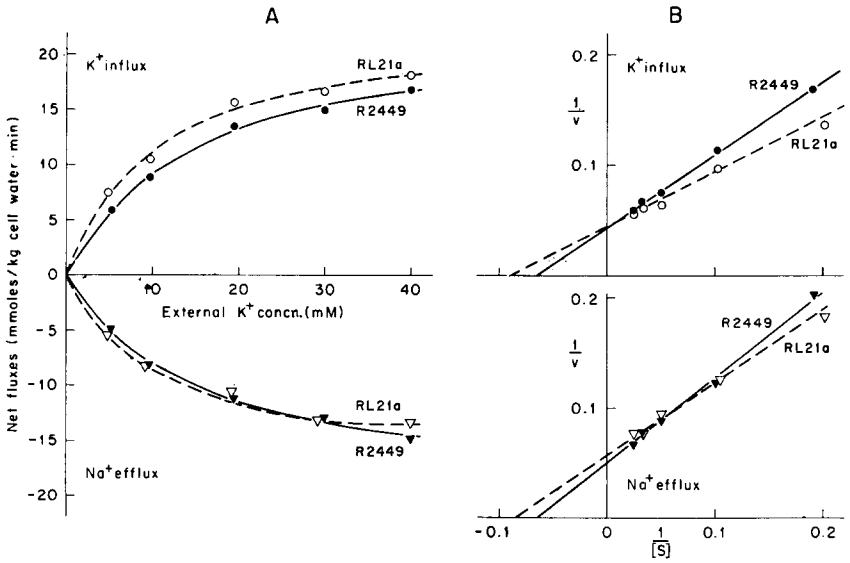


Fig. 1. Dependence of net $K^+$ influx and net $Na^+$ efflux at pH 4.0 on the extracellular $K^+$ concentration for strains RL21a (wild type) and R2449 (mutant). The fluxes are initial rates, calculated from semilog plots of the data as described in METHODS. The points represent average results for three experiments; standard errors (not shown) = $\pm$ 0.06–0.19 mmole/kg cell water·min. Each set of points was computer-fitted to the Michaelis–Menten equation, giving values of $K_{1/2}$, $v_{max.}$ and $\sigma^2$ which are summarized in Table II; and the computed $K_{1/2}$ and $v_{max}$ were then used to draw the curves in A (linear plot) and B (double-reciprocal plot).

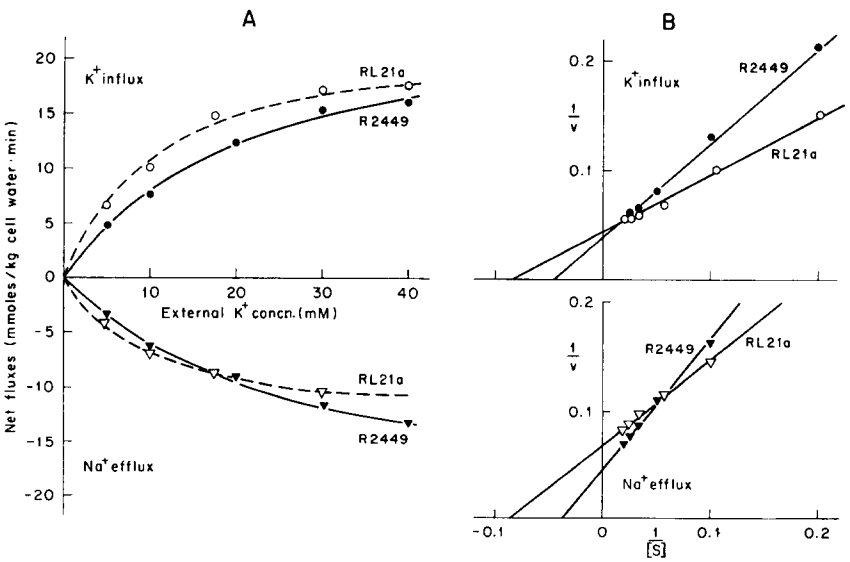


Fig. 2. Dependence of net $K^+$ influx and net $Na^+$ efflux at pH 5.8 on the extracellular $K^+$ concentration, for strains RL21a and R2449. (The wild-type data have been published previously[3], and are included here for comparison with the mutant.) As at pH 4, the fluxes are initial rates, and the points represent average results for three experiments (standard errors = $\pm$ 0.06–0.28 mmole/kg cell water·min). The curves were drawn from computer fits of the Michaelis–Menten equation; see Table II for values of $K_{1/2}$, $v_{max}$, and $\sigma^2$.

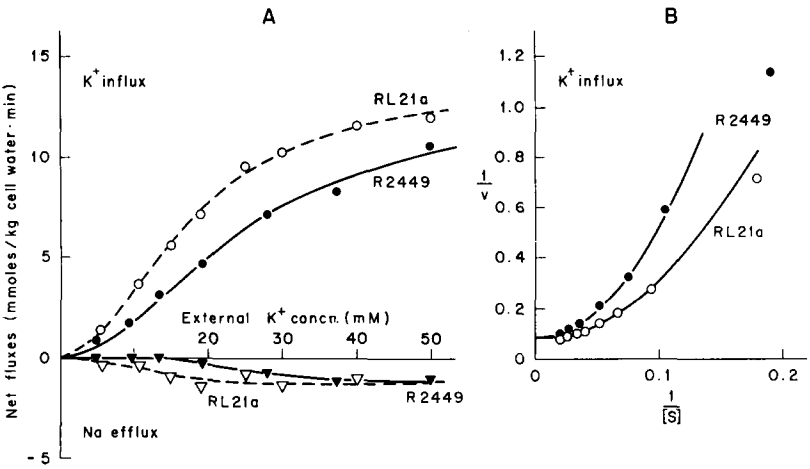


Fig. 3. Dependence of net $K^+$ influx and net $Na^+$ efflux at pH 8.0 on the extracellular $K^+$ concentration, for strains RL21a and R2449. (The wild-type data have been published previously[4].) The fluxes are initial rates for the "transport component", calculated as described in METHODS. The points are average results for three experiments; standard errors = ± 0.07–0.58 mmole/kg cell water·min. The curves for $K^+$ in A (linear plot) and B (double-reciprocal plot) were drawn from computer fits of the data to Eqn. 2 (legend to Table II). Computed values of the 4 parameters in this equation, together with values of $\sigma^2$, are summarized in Table II. Because the $Na^+$ fluxes were too small to be measured very accurately, no attempt was made at fitting this portion of the results by computer; the $Na^+$ curves in A were drawn by eye.

concentration, with $K_{1/2}$'s of 11.1–11.9 mM. From the data in Table II, it seems likely that the three fluxes are also coupled in mutant R2449. At pH 5.8, where the $K_{1/2}$ for $K^+$ influx is raised to 22.3 in the mutant, the $K_{1/2}$'s for $Na^+$ and $H^+$ efflux are raised to 26.6 and 22.8 mM, respectively. And at pH 4, the $K_{1/2}$'s are increased to 15.3 mM for $K^+$ and 15.6 mM for $Na^+$. (The $K_{1/2}$ of $H^+$ efflux has not yet been determined at pH 4.)

The relative magnitudes of the $Na^+$ and $H^+$ effluxes are somewhat different in the mutant, $Na^+$ efflux being increased and $H^+$ efflux decreased relative to wild-type Neurospora (see Figs. 1, 2 and Table II). If the intracellular concentrations of the two cations are the same in both strains, this result would indicate that the relative affinities of the transport system for $Na^+$ and $H^+$ have been altered in R2449. $[Na^+]_i$ at zero time (pH 5.8) averaged 131 ± 5 and 152 ± 5 mmoles/kg cell water in R2449 and RL21a, respectively (Table I), but accurate measurements of intracellular pH have not yet been made.

(3) *$Na^+$ efflux at high pH.* In both wild-type Neurospora and mutant strain R2449, $Na^+$ efflux drops markedly at pH 8 (Fig. 3). Efflux seems to require higher external $K^+$ concentrations in the mutant than in the wild type, but the very low fluxes make it difficult to analyze the data quantitatively. It is not yet clear whether the difference between $K^+$ uptake and $Na^+$ loss is accounted for by the extrusion of $H^+$ at pH 8, as at low pH.

*Analysis of $K^+$ uptake from pH 4 to pH 8 in terms of a single pump*

The clear abnormalities at pH 5.8 and 8 in the mutant strain, together with the smaller but still significant abnormality at pH 4, are difficult to explain in terms

TABLE III

PARAMETER ESTIMATES FOR THE GENERAL $K^+$-UPTAKE MODELS

These values were obtained by fitting the $K^+$ influx data from Figs. 1–3 to the two-site rate equation and the allosteric rate equation (see text), using the MARQUARDT[13] algorithm. For simplicity, in the allosteric case, the ratio $K_S/K_T$ was set at 0 (that is, the $E_S$ conformation was assumed to bind substrate much more tightly than the $E_T$ conformation), and $n$ was set at integral values 2, 3 and 4. All three values of $n$ gave qualitatively similar results; with $n = 4$, $\sigma^2$ (the variance) was slightly lower than with $n = 2$ or 3, so these are the estimates included in the table.

| | *Two-site model* | |
|---|---|---|
| | *RL21a* | *R2449* |
| $K_C$ (mM) | $12.0 \pm 1.2$ | $18.0 \pm 1.5$ |
| $K_{M1}$ (mM) | $16.6 \pm 4.9$ | $48.0 \pm 1.8$ |
| $K_{M2}$ (M) | $6.54 \cdot 10^{-8} \pm 0.28 \cdot 10^{-8}$ | $1.03 \cdot 10^{-7} \pm 0.35 \cdot 10^{-7}$ |
| $k_2\bar{E}$ (mmoles/kg cell water·min) | $24.3 \pm 0.9$ | $24.7 \pm 0.9$ |
| $k_1\bar{E}$ (mmoles/kg cell water·min) | $20.1 \pm 1.5$ | $27.3 \pm 5.3$ |
| $\sigma^2$ | 0.301 | 0.122 |
| | *Allosteric model* | |
| | *RL21a* | *R2449* |
| $L$ | $18.9 \pm 0.4$ | $13.4 \pm 0.3$ |
| $K_R$ (mM) | $10.5 \pm 0.7$ | $16.2 \pm 1.3$ |
| $K_A$ (M) | $1.18 \cdot 10^{-6} \pm 0.22 \cdot 10^{-6}$ | $1.45 \cdot 10^{-6} \pm 0.20 \cdot 10^{-6}$ |
| $k_1nE$ (mmoles/kg cell water·min) | $14.7 \pm 0.4$ | $13.7 \pm 0.5$ |
| $k_2n\bar{E}$ (mmoles/kg cell water·min) | $23.0 \pm 0.5$ | $23.6 \pm 0.8$ |
| $\sigma^2$ | 0.221 | 0.119 |

of a two-pump model, with one system displaying standard Michaelis kinetics at low pH and the other, sigmoid kinetics at high pH. Rather, it has seemed worthwhile to try to devise a single-pump model that would account quantitatively for the changes observed in R2449. By extending an earlier analysis of $K^+$ uptake in wild-type Neurospora, two alternative reaction sequences can be proposed which describe fluxes from pH 4 to 8 in a reasonable manner.

(1) *Two-site model.* According to the first alternative, the cation pump in Neurospora would have two sites: a transport site, with a predominant affinity for $K^+$, and a modifier site, with affinities for both $K^+$ and $H^+$. The reaction sequence may be written as follows:

$$E + S \overset{K_C}{\rightleftharpoons} ES$$

$$E + S \overset{K_{M1}}{\rightleftharpoons} SE$$

$$E + M \overset{K_{M2}}{\rightleftharpoons} ME$$

$$\left.\begin{matrix} SE + S \overset{K_C}{\rightleftharpoons} SES \\ ES + S \overset{K_{M1}}{\rightleftharpoons} SES \end{matrix}\right\} \xrightarrow{k_1} SE + P$$

$$\left.\begin{matrix} ME + S \overset{K_C}{\rightleftharpoons} MES \\ ES + M \overset{K_{M2}}{\rightleftharpoons} MES \end{matrix}\right\} \xrightarrow{k_2} ME + P$$

where $E$ is an element of the transport system, $S$ is the substrate (in this case, extracellular $K^+$), $P$ is the product (intracellular $K^+$) and $M$ is a modifier (extracellular $H^+$). For mathematical convenience, three assumptions are made in deriving the rate equation: (i) The transport site and the modifier sites bind cations in any order and independently; that is, $K_C$ represents the dissociation constant for $K^+$ at the transport site, whether or not the modifier site is filled; and $K_{M_1}$ and $K_{M_2}$ are dissociation constants for $K^+$ and $H^+$ at the modifier site, whether or not the transport site is filled. (ii) Both sites must be filled in order for transport to occur; only $SES$ and $MES$ can react to form the product. (iii) Finally, translocation of the ternary complexes $SES$ and $MES$ across the membrane, breakdown of the complexes to form product, and return of the system to the initial condition are all lumped together and designated by rate constants $k_1$ and $k_2$. The overall reactions of $SES$ and $MES$ are assumed to be slow ($k_1$ and $k_2$ assumed to be small), so that the binding reactions can be considered to be at equilibrium.

Under these conditions, the rate equation for the two-site model is:

$$v = \frac{\frac{K_{M1}}{K_{M2}} k_2 \bar{E} [M] [S] + k_1 \bar{E} [S]^2}{K_C K_{M1} + \frac{K_C K_{M1}}{K_{M2}} [M] + \frac{K_{M1}}{K_{M2}} [M] [S] + (K_C + K_{M1}) [S] + [S]^2}$$

where $v$ is the initial velocity of uptake, $[S]$ and $[M]$ are the concentrations of substrate and modifier, and $\bar{E}$ is the sum of all forms of the enzyme (assumed to be constant). Accordingly, $k_2\bar{E}$ is the maximal velocity at low pH, when all of the system is in the $MES$ form, and $k_1\bar{E}$ is the maximal velocity at high pH, with all of the system in the $SES$ form.

Table III gives the results of a computer fit of this model to all of the data (pH 4, 5.8 and 8) from mutant strain R2449 and wild-type Neurospora. Two main differences are apparent: (a) $K_C$, the dissociation constant for $K^+$ at the transport site, is approx. 50 % higher in the mutant (18.0 mM) than in the wild type (12.0 mM). [These values agree reasonably well with $K_{\frac{1}{2}}$'s determined directly from the pH 4 data using the Michaelis–Menten equation: 15.3 and 11.1 mM, respectively (Table II).] (b) The dissociation constants for both $K^+$ ($K_{M_1}$) and $H^+$ ($K_{M_2}$) at the modifier site are increased in the mutant: from 16.6 to 48.0 mM for $K^+$, and from $6.5 \cdot 10^{-8}$ to $1.0 \cdot 10^{-7}$ M for $H^+$. Since the increase for $K^+$ (3-fold) is greater than the increase for $H^+$ (1.5-fold), the net result is that the mutant modifier site will tend to bind $H^+$ better than $K^+$, relative to the wild-type site; and the mutant should therefore require higher pH's to bring about the shift from one-site kinetics to two-site kinetics. As shown in Table IV, Hill plots of mutant and wild-type data give results that are consistent with this idea. Values of $n$ (the slope of the Hill plot, related to the number of sites[14]) were determined as a function of pH for both strains; wild-type Neurospora shifts from $n = 1.06$ at pH 4 to $n = 2.01$ at pH 8, while R2449 shows a conspicuously smaller change, from 1.03 at pH 4 to 1.52 at pH 8.

(2) *Allosteric model.* As an alternative to the two-site mechanism, it is possible to interpret $K^+$ uptake by both mutant and wild-type strains in terms of an allosteric transport system in which the carrier is composed of multiple subunits, each with an active site for $K^+$. The carrier is assumed to exist in two conformations with different

TABLE IV

ESTIMATES OF $n$ FROM HILL PLOTS AT pH 4, 5.8 AND 8

These values were obtained by fitting the $K^+$ influx data from Figs. 1–3 to the Hill equation, $v = v_{max}[S]^n/([K_{½}]^n + [S]^n)$, using the MARQUARDT[13] algorithm. All values are mean ± S.E.

| *pH* | *RL21a* | *R2449* |
|---|---|---|
| 4.0 | 1.06 ± 0.26 | 1.03 ± 0.20 |
| 5.8 | 1.32 ± 0.19 | 1.16 ± 0.24 |
| 8.0 | 2.01 ± 0.12 | 1.52 ± 0.19 |

affinities for $K^+$; at high pH, interaction with $K^+$ shifts the equilibrium progressively toward the high-affinity conformation, thus generating a sigmoid curve of transport as a function of $[K^+]_0$, while at low pH, the equilibrium is shifted in the same direction by interaction with $H^+$, and transport follows a standard Michaelis-type rectangular hyperbola. By analogy with the equations of MONOD *et al.*[15], the rate equation for such a system is:

$$v = \frac{[S]K_T{}^n(K_R + S)^{n-1}[(k_1 - k_2)n\overline{E}K_A{}^n + k_2n\overline{E}(K_A + A)^n]}{K_T[(K_R + S)(K_T + S)]^n + L[K_RK_A(K_T + S)]^n}$$

where $[S]$ is again the concentration of substrate (extracellular $K^+$); $[A]$ is the concentration of activator ($H^+$); $K_R$ and $K_T$ are equilibrium constants for the interaction of substrate with $E_R$ and $E_T$ (the two conformations of the enzyme); $K_A$ is the equilibrium constant for the interaction of activator with $E_R$ (activator is assumed not to bind to $E_T$); $n$ is the number of subunits; $L$ is the ratio $E_T/E_R$; and $k_1$ and $k_2$ are rate constants for the breakdown of the $E_R{\cdot}S$ and $HE_R{\cdot}S$ complexes. For simplicity, it is assumed that $E_T$ is not enzymatically active although it can still bind substrate.

Table III shows the results of a computer fit of the allosteric rate equation, and again two differences can be seen between the mutant and wild-type strains: (i) As in the previous model, $K_R$, the dissociation constant of $K^+$ from the active conformation of the enzyme, is approx. 50% higher in R2449 (16.2 mM) than in RL21a (10.5 mM). (ii) In addition, $L$ (the ratio of the two conformations, $E_T/E_R$) is altered in the mutant (13.4) compared with the wild type (18.9). This difference—the fact that relatively more of the mutant enzyme tends to exist in the active $E_R$ conformation—can be correlated with the generally lower values of $n$ at pH 5.8 and pH 8 (Table III).

## DISCUSSION

From the analysis presented above, we conclude that $K^+$ uptake from pH 4 to pH 8 can be accounted for quantitatively by a single transport system in wild-type Neurospora, with distinct alterations of the system in mutant strain R2449. Under both the allosteric and the two-site models, the higher $K^+$ requirement of the mutant would reflect an increase in the $K_{½}$ of the transport site(s). The abnormal pH dependence of the mutant would come from an altered equilibrium between the two

conformations of the carrier, in the allosteric model, or from altered affinities of the modifier site for $H^+$ and $K^+$, in the two-site model. Further experiments will be necessary to distinguish between these possibilities. In the meantime, it is interesting to note that multisite kinetics have been observed for cation transport in other organisms (yeast[16,17], frog muscle[18–20] and human red blood cells[21,22]), and may turn out to be a general feature of this class of transport systems.

The models described in this paper deal only with the kinetics of potassium influx and have not yet been extended to cover the coupled efflux of $Na^+$ and $H^+$. This treatment is sufficient for mutant R2449 because it, like mutants that have been reported for *E. coli*[23] and *S. faecalis*[24], is defective mainly in influx. Other mutants of *E. coli*[25–27], *S. faecalis*[28,29], *Bacillus subtilis*[30] and Neurospora (C. W. SLAYMAN, unpublished results) have been found whose primary defects are in the extrusion of $K^+$, $Na^+$ or $H^+$; and at least in Neurospora, these efflux mutants are not linked to R2449 (C. W. SLAYMAN, unpublished results). Cation transport systems are almost certainly large and complex, however [the apparent molecular weight of Na,K ATPase, extracted from guinea pig brain microsomes with Lubrol and chromatographed on agarose, is 670000 (ref. 31)], and may well contain several subunits, each coded for by a separate gene. Under this hypothesis, mutations affecting primarily one portion of the system may still cause detectable secondary alterations of another portion, for example, the reduction in R2449 of $H^+$ efflux relative to $Na^+$ efflux, which needs to be investigated in further detail.

ACKNOWLEDGMENTS

This work was supported by Public Health Service Research Grant GM 15761 and by a Public Health Service Research Career Development Award (No. GM 20163). Mr. Robert Kopsack provided expert technical assistance throughout the experiments.

REFERENCES

1 C. W. SLAYMAN AND E. L. TATUM, *Biochim. Biophys. Acta*, 88 (1964) 578.
2 C. W. SLAYMAN AND E. L. TATUM, *Biochim. Biophys. Acta*, 102 (1965) 149.
3 C. L. SLAYMAN AND C. W. SLAYMAN, *J. Gen. Physiol.*, 52 (1968) 424.
4 C. W. SLAYMAN AND C. L. SLAYMAN, *J. Gen. Physiol.*, 55 (1970) 758.
5 F. M. HAROLD, R. L. HAROLD AND A. ABRAMS, *J. Biol. Chem.*, 240 (1965) 3145.
6 F. M. HAROLD AND J. R. BAARDA, *J. Bacteriol.*, 91 (1966) 2257.
7 P. V. BENKO, T. C. WOOD AND I. H. SEGEL, *Arch. Biochem. Biophys.*, 122 (1967) 783.
8 M. GRENSON, M. MOUSSET, J. M. WIAME AND J. BECHET, *Biochim. Biophys. Acta*, 127 (1966) 325.
9 M. GRENSON, *Biochim. Biophys. Acta*, 127 (1966) 339.
10 J. J. GITS AND M. GRENSON, *Biochim. Biophys. Acta*, 135 (1967) 507.
11 M. L. PALL, *Biochim. Biophys. Acta*, 173 (1969) 113.
12 C. W. SLAYMAN AND E. L. TATUM, *Biochim. Biophys. Acta*, 109 (1965) 184.
13 D. W. MARQUARDT, *J. Soc. Ind. Appl. Math.*, 11 (1963) 431.
14 J. WYMAN, *J. Am. Chem. Soc.*, 89 (1967) 2202.
15 J. MONOD, J. WYMAN AND J.-P. CHANGEUX, *J. Mol. Biol.*, 12 (1965) 88.
16 W. McD. ARMSTRONG AND A. ROTHSTEIN, *J. Gen. Physiol.*, 48 (1964) 61.
17 W. McD. ARMSTRONG AND A. ROTHSTEIN, *J. Gen. Physiol.*, 50 (1967) 967.
18 R. D. KEYNES AND R. C. SWAN, *J. Physiol. London*, 147 (1959) 591.
19 R. D. KEYNES, *J. Physiol. London*, 178 (1965) 305.
20 L. J. MULLINS AND A. S. FRUMENTO, *J. Gen. Physiol.*, 46 (1963) 629.
21 J. R. SACHS, *J. Clin. Invest.*, 46 (1967) 1433.
22 J. R. SACHS AND L. G. WELT, *J. Clin. Invest.*, 46 (1967) 65.
23 R. DAMADIAN, *J. Bacteriol.*, 95 (1968) 113.

24 F. M. HAROLD AND J. R. BAARDA, *Biochemistry*, 6 (1967) 3107.
25 M. LUBIN AND H. L. ENNIS, *Biochim. Biophys. Acta*, 80 (1964) 614.
26 T. GÜNTHER AND F. DORN, *Z. Naturforsch.*, 21b (1966) 1082.
27 B. LUBOCHINSKY, J. MEURY AND J. STOLKOWSKI, *Compt. Rend.*, 258 (1965) 5106.
28 F. M. HAROLD, R. L. HAROLD, J. R. BAARDA AND A. ABRAMS, *Biochemistry*, 6 (1967) 1777.
29 F. M. HAROLD, J. R. BAARDA AND E. PAVLASOVA, *J. Bacteriol.*, 101 (1970) 152.
30 D. B. WILLIS AND H. L. ENNIS, *J. Bacteriol.*, 96 (1968) 2035.
31 F. MEDZIHRADSKY, M. H. KLINE AND L. R. HOKIN, *Arch. Biochem. Biophys.*, 121 (1967) 311.