A Potassium-Proton Symport
in *Neurospora crassa*

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ABSTRACT   Combined ion flux and electrophysiological measurements have been used to characterize active transport of potassium by cells of *Neurospora crassa* that have been moderately starved of K^+ and then maintained in the presence of millimolar free calcium ions. These conditions elicit a high-affinity ($K_{1/2} = 1–10 \mu M$) potassium uptake system that is strongly depolarizing. Current-voltage measurements have demonstrated a K^+-associated inward current exceeding (at saturation) half the total current normally driven outward through the plasma membrane proton pump. Potassium activity ratios and fluxes have been compared quantitatively with electrophysiological parameters, by using small (~15 μm diam) spherical cells of *Neurospora* grown in ethylene glycol. All data are consistent with a transport mechanism that carries K ions inward by cotransport with H ions, which move down the electrochemical gradient created by the primary proton pump. The stoichiometry of entry is 1 K ion with 1 H ion; overall charge balance is maintained by pumped extrusion of two protons, to yield a net flux stoichiometry of 1 K^+ exchanging for 1 H^+. The mechanism is competent to sustain the largest stable K^+ gradients that have been measured in *Neurospora*, with no direct contribution from phosphate hydrolysis or redox processes. Such a potassium-proton symport mechanism could account for many observations reported on K^+ movement in other fungi, in algae, and in higher plants.

INTRODUCTION

The ability to generate and sustain high intracellular levels of potassium, against low extracellular concentrations of that ion, is a well-defined property of almost all living cells. This property is especially prominent in fungi and plants, for which normal extracellular concentrations of the ion can be in the micromolar range. Concentration ratios, $[K^+]_i/[K^+]_o$, as high as 2.5×10^4 have been reported for yeast (Borst-Pauwels, 1981) in the steady state, and ratios in the range of $10^3–10^4$ are achieved in almost all organisms (Tromballa, 1980; Cheeseman and

Hanson, 1980; Hauer et al., 1981; van Brunt et al., 1982). The transport mechanisms that account for such large concentration ratios have many common features in all plants and fungi. At the concentration extremes, K^+ uptake is thermodynamically "active," with net flux occurring against the total electrochemical potential difference for K^+, and it is strongly inhibited by agents blocking either energy metabolism or the major plasma membrane (H^+-transporting) ATPase (Peña, 1975; Leonard and Hotchkiss, 1976; Cocucci et al., 1980; Cheeseman et al., 1980; Tromballa, 1981; Hauer et al., 1981; van Brunt et al., 1982; Rahat and Reinhold, 1983). In most cases that have been specifically checked, uptake of K^+ occurs in ~1:1 exchange for H^+ and leads to detectable cytoplasmic alkalinization (Conway and O'Malley, 1946; Conway and Downey, 1950; Tromballa, 1978; Ogino et al., 1983). Finally, in the whole range of cell types, growth or preincubation under conditions of potassium starvation greatly enhances both the velocity and affinity of K^+ transport, with apparent K_m values falling to the neighborhood of 10 μM (Epstein, 1966; Cheeseman et al., 1980; Hauer et al., 1981).

Interpretation of the mass of K^+ transport data on plants and fungi is somewhat bewildering, however; this is due in part to the study of a very wide range of species and of tissues within any one species, and in part to the intrinsic difficulty of making all relevant transport measurements (isotope flux, net flux, electrical, metabolic) on a single cell or tissue type. The most persistent notion, which arose from reasoning by analogy with the sodium pump in animal cells (Hodges, 1976), has been that K^+ uptake in plants and fungi is chemically coupled to the active efflux of another cation—now usually regarded as H^+—through an ATPase pump (Poole, 1978; Leonard, 1982). Experimental support has come (a) from repeated observations that membrane-bound ATPases in plants and fungi are stimulated by K ions (see, e.g., Leonard and Hotchkiss, 1976; Perlin and Spanswick, 1981; Sze and Churchill, 1981; Pomeroy and McMurchie, 1982); (b) from the finding that K^+ translocation occurs in liposomes containing functional yeast plasma membrane ATPase (Villalobo, 1984); and (c) from reports that extrinsic factors, such as aging, hormones, or toxins, modulate proton pumping and K^+ uptake in parallel (Lin and Hanson, 1974; Marré et al., 1974; Lin, 1979; Pitman et al., 1975; Tromballa, 1978). The notion has not really solidified, however, because of several anomalous results. (a) Cation stimulation of certain ATPases, such as that in the Neurospora plasma membrane (Bowman and Slayman, 1977), is a salt effect rather than a specific K^+ effect. (b) Maximal stimulation is usually small (1.2–3-fold) and nonsynergistic. (The Na^+/K^+ ATPase of animal cells, by comparison, hydrolyzes ATP ~30-fold faster in the presence of Na^+ [10–100 mM] plus K^+ [10–20 mM] than in the presence of either cation alone [Dunham and Glynn, 1961]; the H^+/K^+ ATPase of gastric mucosa hydrolyzes >50-fold faster with 1 mM K^+ in pH 7.4 buffer, than without the K^+ [Wallmark et al., 1980].) (c) Parallel variation of K^+ transport flux and K^+-activated ATPase levels breaks down under many conditions (Jensén et al., 1983). (d) In proteoliposomes containing yeast plasma membrane ATPase, potassium stimulation of proton pumping is exerted by the addition of K^+ to the ATPase side of the membrane (Vara and Serrano, 1982).

Another simple mechanism that could be imagined for the observed 1:1 coupling between K^+ influx and H^+ efflux is actual exchange of the two species on a secondary active transport system, not directly coupled to ATP hydrolysis. This was suggested explicitly to account for anomalous K^+ transport during electric pulsing of *Nitella* (Barr et al., 1977; Ryan et al., 1978). It was also initially postulated to account for dithioerythritol enhancement of K^+/H^+ exchange in corn roots (Lin and Hanson, 1976), but that picture has recently been complicated by dissection of two current-carrying systems for K^+ uptake (Cheeseman et al., 1980).

In fact, a survey of ion balance studies on algae and higher plants shows that net K^+ uptake can be neutralized under different conditions by Na^+ efflux, Cl^- influx, and HCO_3^- influx, as well as by H^+ efflux (Jackson and Adams, 1963; Jackson and Stief, 1965; Poole, 1966; Kylin, 1966; Findlay et al., 1969; Nobel, 1969; MacRobbie, 1970), but there is no evidence for direct coupling except in the case of protons. Such diversity leads to the reasonable suggestion that a changing ensemble of active and passive transport systems may operate for potassium and other inorganic ions, so the composition of flux balance depends upon "physiologic need." For plant cells, however, a quantitative demonstration of even passive transport systems has proven illusory, and is unequivocal only under special conditions, such as calcium leaching of algal cells (Hope and Walker, 1960), triggering of gated Cl^- or K^+ conductances (Kishimoto, 1964; Findlay, 1982; Findlay and Coleman, 1983), or physical isolation of bona fide K^+ channels by means of the patch-electrode technique (Schroeder et al., 1984; Moran et al., 1984).

The experiments described below on *Neurospora crassa* were begun as a study of the kinetic effects of potassium starvation, and were undertaken in part to reconcile (*a*) the published description (Slayman and Slayman, 1968; Slayman and Slayman, 1970) of a high-velocity, low-affinity K^+ transport system seen without added Ca ions with (*b*) a low-velocity scavenger mechanism discovered by H. Pfrüner (unpublished results) several years ago in experiments with 0.1–10 mM added extracellular calcium. The problem of making all relevant measurements on the same cell type was resolved with spherical cells of *Neurospora* obtained by incubating conidiospores in growth medium containing 3.2 M ethylene glycol (Bates and Wilson, 1974; Blatt and Slayman, 1983). Potassium-starved *Neurospora* maintained in millimolar extracellular calcium displayed high-affinity uptake of K^+ associated with stoichiometric extrusion of protons. The mechanism proved to be strongly electrophoretic (depolarizing), such that two charges must enter the cells with each potassium ion. This crucial finding, along with several other observations, strongly implicates a 1:1 K^+-H^+ cotransport mechanism, which would thereby closely resemble the proton-linked amino acid and sugar cotransport systems already described in *Neurospora* (Sanders et al., 1983; Slayman and Slayman, 1974). The existence of a K^+-H^+ symport mechanism was anticipated by Bakker and Harold (1980), working with *Streptococcus faecalis*, and has recently been postulated by Boxman et al. (1984) for *Saccharomyces*. A preliminary report of these experiments on *Neurospora* has also been published (Blatt et al., 1984).

MATERIALS AND METHODS

Growth and Preparation of Cells

The wild-type strain RL21a of *Neurospora crassa* was used throughout these experiments. The general methods for growing and handling the cells have been described previously (Slayman and Tatum, 1964; Slayman and Slayman, 1968), but in the present experiments potassium-depleted cells for flux measurements were grown in two different ways. So-

TABLE I

Compositions of Growth Media* and Flux Buffers

	AmVM‡	NaVM	CaMES	StDMG
Na⁺	0ˢ	61.8	0	25
K⁺	0.3	0.3	0.001–0.2 as needed	
NH₄⁺	40	25	0	0
Ca⁺⁺	0.1	0.1	1.5	1
Mg⁺⁺	0.8	0.8	0	0
Cl⁻	0.5	0.3	—ˡ	2
NO₃⁻	0	25	0	0
SO₄⁻	0.8	0.8	0.005–0.1 added with K⁺ˡ	0
Phosphate	15	36.8	0	0
Citrate	8.4	8.4	0	0
MES¹	0	0	10	0
DMG¹	0	0	0	20
Sucrose (%)	2 or 0**	2 or 0	0	0
Glucose (%)	0 or 1**	0 or 1	1 or 0	1 or 0
EG‡‡				

Concentrations are given in millimolar or percent weight per volume.

* Growth media here were modified from the "N" minimal medium of Vogel (1956), and contained the same heavy-metal trace elements and biotin. The flux buffers did not contain trace elements or biotin.

‡ Except as specifically noted in the text or figure legends, all solutions used in these experiments were buffered at pH 5.80 ± 0.05. Heading abbreviations are defined in the text.

ˢ Components designated by "0" were simply omitted. Actual solutions concentrations achieved were 5–10 μM for Na⁺, 1–2 μM for Ca⁺⁺, and <1 μM for K⁺.

ˡ In a few experiments KCl, rather than K_2SO_4, was added to raise [K⁺]ₒ.

¹ MES = 2-N-morpholinoethanesulfonic acid; DMG = 3,3′-dimethylglutaric acid.

** Sucrose media were used to grow shaking-culture cells; glucose media were used to grow spherical cells. Flux buffers contained glucose for shaking-culture cells and no sugar or added carbon source for the spherical cells.

‡‡ EG was added at a concentration (vol/vol) of 18%, to medium AmVM for growing spherical cells, but to the buffer CaMES only for rinsing the spherical cells.

called shaking-culture cells were prepared by germinating conidia in a low-K⁺ minimal medium based on Vogel's medium (Vogel, 1956), and buffered with citrate and either ammonium phosphate (AmVM) or sodium phosphate (NaVM) containing 0.3 mM KCl (see Table I). The conidia were inoculated at 3 × 10⁶/ml, and the suspensions were maintained on a reciprocating shaker (110 strokes/min) at 25°C for 11–13 h. By the end of this time, extracellular [K⁺] had fallen below 20 μM and intracellular [K⁺] was diluted to ~100 mM, as described by Ramos and Rodriguez-Navarro (1985). Cells obtained by this technique are unbranched filaments 3–5 μm in diameter and 60–150 μm long.

Because such cells are too slender for ready penetration with microelectrodes, correlated flux and electrophysiological experiments were carried out on spherical cells (Blatt and Slayman, 1983) grown in ethylene glycol (EG). Conidia were inoculated, again at 3×10^6/ml, into AmVM containing 15% EG (vol/vol, 2.7 M) and supplemented with 1% glucose (instead of the usual 2% sucrose; see Table I). The cells were maintained at 25°C for 3 d on a rotary shaker (20 cycles/min) or tube rotator (10–15 cycles/min). Before use, the cells needed to be washed of EG, and in order to accomplish this without either bursting the cells or allowing subsequent germination, the following procedure was adopted. The growing cells were harvested by filtration, and then rinsed and resuspended in flux buffer (see below) containing 18% EG, but lacking glucose or other potential carbon sources. The EG was then diluted 10-fold by adding EG-free flux buffer gradually, over a period of 3 h. At the end of that time, cells for flux experiments were harvested again and resuspended in EG- and glucose-free buffer, while cells for electrical experiments were diluted into the recording chamber and maintained in a flowing stream of that same buffer (Blatt and Slayman, 1983).

The compositions of the major growth media and buffer solutions used in these experiments are given in Table I. The preferred growth medium was AmVM, and the preferred flux buffer was CaMES, since both intracellular and extracellular Na^+ was found to interfere with K^+ uptake, as had previously been shown for *Saccharomyces* (Armstrong and Rothstein, 1967). All reagents used in these experiments were reagent-grade chemicals obtained from either J. T. Baker Chemical Co. (Phillipsburg, NJ), Calbiochem-Behring (American Hoechst Corp., La Jolla, CA), or Sigma Chemical Co. (St. Louis, MO).

Flux Experiments

Shaking-culture cells were harvested, rinsed thoroughly (usually three times with 10 ml of distilled water), resuspended in flux buffer with 1% glucose (at a density of 0.05–0.5 mg dry weight/ml), and preincubated for 5–10 min in a water-bath shaker (25°C). Then KCl or K_2SO_4 was added to give the desired starting $[K^+]_o$, and 2–5-ml aliquots of the suspension were removed at intervals (usually at ~15, 30, 60, and 90 s) and syringe-filtered through a Millipore membrane (type RA, Millipore Corp., Bedford, MA). The filtrate was collected and analyzed for chemical potassium by means of a flame absorption spectrophotometer (model 560, Perkin-Elmer Corp., Norwalk, CT) and, in studies of unidirectional influx, for $^{42}K^+$ by means of a Nuclear Chicago auto-gamma counter (G. B. Searle Co., Chicago, IL). Shaking-culture cells handled in the manner described above formed very homogeneous suspensions; the actual cell mass corresponding to each solution volume analyzed was obtained from parallel samples, which had been collected on Millipore filters, rinsed with distilled water, dried overnight at 95°C, and weighed. Thus, primary flux data were obtained per unit dry weight, and cytoplasmic volumes were calculated from the ratio intracellular water/dry weight = 2.54, determined previously (Slayman and Tatum, 1964; Slayman and Slayman, 1968). Corresponding surface areas were calculated from the average cylindrical diameter of 4 μm, giving a surface/volume ratio of 10^4 cm^2/cm^3.

Spherical cells, washed free of EG, were resuspended at 5×10^5 cells/ml in flux buffer and preincubated for 30–90 min with aeration (via a fine-tipped plastic pipette), before starting the flux measurements. (Trial experiments had shown that at least 30 min of aeration was required to maximize the transport rates.) After preincubation, the cells were again harvested, resuspended at $1–5 \times 10^6$/ml in air-saturated buffer, and assayed for potassium influx during maintained aeration. Flux assays were carried out exactly as described above for shaking-culture cells, but in flux buffer without glucose. Determination of cell volume, however, was not so simple because the cell diameters varied greatly,

and the intracellular water/dry weight ratio differed considerably from that for shaking-culture cells. (Three separate estimates of this ratio, using [^{14}C]sorbitol as the label for extracellular space, gave values near 4 kg water/kg dry weight.) Therefore, the cell volumes and suspension densities were calculated from diameter measurements and cell counts made on a Neubauer chamber (at magnifications of 100 and 400) for each experiment. A very rough average diameter for the spherical cells was 15 ± 3 μm (mean ± 1 SD), which implies a surface/volume ratio of 4 × 10^3 cm^2/cm^3.

K^+/H^+ Exchange

Net uptake of potassium and net release of protons were followed simultaneously (see, e.g., Fig. 5) by means of ion-sensitive electrodes. A commercial pH-combination electrode (model 4098-M30, A. H. Thomas Co., Swedesboro, NJ) was used, but was modified by having the reference bridge filled with saturated NaCl, in order to prevent potassium leakage into the low-K$^+$ suspensions. K$^+$-sensitive electrodes were fabricated by sealing a valinomycin-impregnated polyvinyl chloride membrane over the ends of pulled capillary tubes (1.5 mm o.d.) and inserting an Ag/AgCl half-cell in 0.1 M KCl (Ballarin-Denti et al., 1984). The pH electrode was connected to a pH meter (model 25, Radiometer America, Westlake, OH) and the K$^+$ electrode was connected to an electrometer amplifier (model FD223, W-P Instruments, Inc., New Haven, CT). Outputs from both amplifiers were led to a two-pen strip-chart recorder (Servoriter II, Texas Instruments Corp., Dallas, TX). For these experiments, a special buffer solution was used: 10 mM BES (2-[bis(2-hydroxyethyl)amino]ethanesulfonic acid) titrated to approximately pH 5 with CaOH and containing 2 mM added CaCl$_2$. This low-pH condition served both to minimize possible errors from CO$_2$ absorption and to increase the sensitivity of H$^+$ measurement, by reducing buffer capacity.

Microelectrodes

Multibarreled microelectrodes, for simultaneous recording of membrane potential, voltage-clamping, and ionophoresis or sensing of cytoplasmic ion activities, were fabricated as described previously (Blatt and Slayman, 1983). In the present experiments, (potassium) acetate was chosen as the filling electrolyte, since most other anions were found to damage the spherical cells of *Neurospora* (Blatt and Slayman, 1983). Low concentrations—50 mM (or occasionally 100 mM)—were used, in order to keep the cytoplasmic cation composition of punctured cells close to that of ordinary K$^+$-starved cells (see above), and were prepared by titrating acetic acid to pH 7 with potassium hydroxide. Control experiments were then carried out to determine whether the salt concentration in the pipette solution would alter membrane electrical properties, under the conditions of these experiments. A summary of the results is shown in Fig. 1, where the membrane current during potassium uptake is plotted against the concentration of potassium acetate (KOAc) in the microelectrode. Over the range 50–200 mM, the electrode salt concentration had no effect on the *I-V* data, although at lower levels (≤20 mM) enhanced currents were seen, presumably as an additional starvation effect. (However, the fact that higher KCl electrodes did not depress K$^+$ uptake indicates that the starvation procedure, rather than the actual cytoplasmic concentration, is essential to evoke the high-affinity transport system.)

Recording Arrangements

Electronic and mechanical conditions for recording from spherical cells were similar to those reported previously (Blatt and Slayman, 1983). The ion-sensing barrel of the microelectrodes was connected to an ultrahigh-impedance amplifier (WPI model FD223). Voltage and current electrodes were connected to electrometers fitted with a current-

injection circuit (WPI model M701, with 10^{12} Ω input impedance), which led to an oscilloscope (model 5113, Tektronix, Inc., Beaverton, OR), a chart recorder, and a microprocessor-controlled voltage clamp. *I-V* curves were plotted from paired currents and voltages, measured as the voltage clamp was driven through a series of brief (100 ms) pulse steps scanning the range −150 to about −350 mV. Using some caution (Blatt, M. R., A. Rodriguez-Navarro, and C. L. Slayman, manuscript in preparation), the membrane current associated with potassium transport could be calculated as the difference between two *I-V* curves, obtained in the presence and absence of extracellular K^+. Current densities were calculated on the basis of spherical geometry, with diameters measured by a calibrated ocular micrometer.
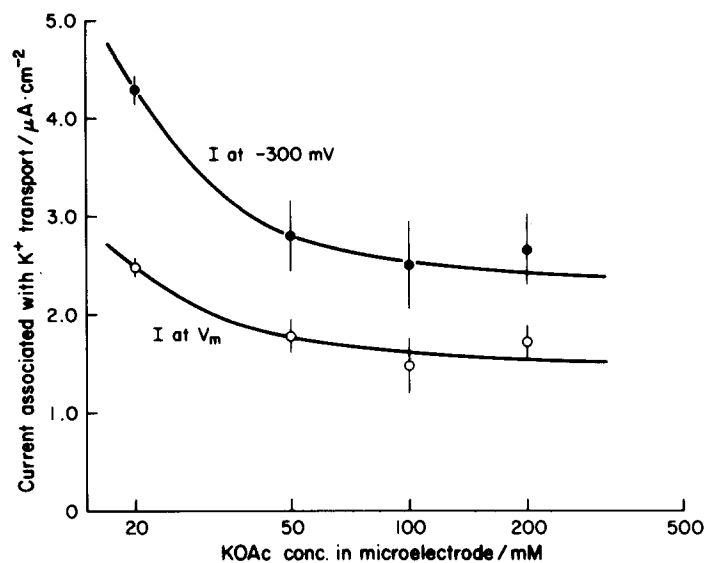

FIGURE 1. Influence of electrode-filling solution upon high-affinity potassium transport in spherical cells of *Neurospora*. Double-barreled microelectrodes were filled with different concentrations of potassium acetate. Current-voltage experiments were similar to that in Fig. 6, with the difference current measured at two voltages: −300 mV and the minimal membrane potential (V_m) obtained upon addition of 50 μM K_2SO_4 to the medium. Cells were grown 3 d in (EG)AmVM, and measured in CaMES. The average cytoplasmic K^+ concentration in such cells, before impalement, was ~80 mM. The values plotted are averages for three to four cells.

Cells to be used in electrical experiments were spread on a polylysine-coated coverslip. After 10 min had been allowed for cells to settle and adhere, the coverslip was inverted and sealed onto the recording chamber, on the stage of a conventional phase-contrast microscope (model ZETOPAN, AO-Reichert, Vienna, Austria). Individual cells for impalement were retrieved by a suction pipette, which was later withdrawn, leaving the punctured cell supported only by the recording electrode.

During all electrical measurements, the recording solution constantly perfused the chamber (exchanging at ~10 chamber vol/min). Rapid changes of solution (addition and subtraction of K^+) were accomplished by means of a large capillary tube (300 μm diam) placed 100 μm upstream from the impaled cell and lateral to it (analogous to the procedure of Yellen, 1982; see also the diagram of Fig. 5A, below). Flow out of the capillary was

matched to the chamber flow (to avoid turbulence), and the composition of the capillary solution was preset for each test. The solution change was then accomplished by moving the capillary laterally, to place it directly upstream from the impaled cell (see Fig. 5A). By this means, solution shifts at the impaled cell were easily completed in 10–50 ms (monitored by changes of tip junction potential in free microelectrodes).

RESULTS

Background Description of High-Affinity K^+ Transport

The kinetic characteristics of potassium transport in wild-type *Neurospora* mycelium vary greatly, according to the conditions under which mycelium has been prepared. For steady state exchange of potassium into cells of normal K^+ content (~180 mmol/kg cell water = 180 mM), Slayman and Tatum (1965) found a maximal velocity (J_{max}) of 20 mM/min and a $K_{1/2}$ for extracellular potassium of 1 mM, using buffers that contained 25 mM sodium but no added calcium (free $Ca^{++} < 10$ μM). Slayman and Slayman (1968) found net uptake of potassium, in cells grown 16 h on limiting potassium ($[K^+]_i \simeq 60$ mM) and maintained in low-calcium solutions, to have nearly the same maximal velocity (23 mM/min) but an ~10-fold-larger $K_{1/2}$ (11.8 mM). Subsequently, H. Pfrüner (unpublished experiments) showed that 1 mM Ca^{++} added to the incubation buffer greatly reduced K^+/K^+ exchange flux (saturating fluxes of 2–4 mM/min), but had little effect on net K^+ uptake. Finally, Ramos and Rodriguez-Navarro (1985) demonstrated that, in the presence of Ca^{++}, the $K_{1/2}$ for uptake of rubidium by K^+-starved cells could fall to the range of 1–10 μM, depending on how low $[K^+]_i$ had dropped during starvation. Both steady state K^+/K^+ exchange and net K^+ (or Rb^+) uptake were found to be metabolically dependent, being blocked by millimolar cyanide, irrespective of the magnitude or direction of the existing concentration gradient for potassium.

In the present experiments, net potassium uptake by K^+-starved (but calcium-replete) cells was also found to follow simple saturation curves (Fig. 2), with J_{max} values of 10–15 mM/min (slightly more than half the maximal fluxes reported previously in the absence of added calcium) and with micromolar $K_{1/2}$ values. Carbon starvation, a maneuver required to prepare the spherical cells (see below), affects both kinetic parameters, lowering J_{max} from 14.4 ± 1.0 to 10.1 ± 0.4 mM/min in Fig. 2, and raising $K_{1/2}$ from 2.0 ± 0.5 to 6.7 ± 1.0 μM.

Elementary Transport Properties of Spherical Cells

Spherical cells of *Neurospora*, grown on limiting potassium, contained 230–290 nmol K^+/mg dry wt, assayed photometrically, and cytoplasmic K^+ activities of ~60 mM, assayed with ion-sensitive microelectrodes. These values, together with the surface/volume data (see Materials and Methods), are consistent with an activity coefficient of 0.8 for K^+ in cytoplasm, which is tolerably close to the value in free solution. Absolute transport rates, for any given test condition with spherical cells, were almost always smaller than the corresponding rates observed with shaking-culture cells, when fluxes were expressed in units of millimoles per kilogram dry weight. This discrepancy might be related either to the culture conditions for the two cell types or to the different surface/volume ratios (see Materials and Methods). It was essentially abolished by expressing fluxes on the

basis of unit membrane area (i.e., $pmol \cdot cm^{-2} \cdot s^{-1}$), so that convention was adopted for all further experiments. As in all other published results on potassium transport in *Neurospora*, high-affinity flux into the spherical cells was blocked by respiratory inhibitors such as cyanide and azide.
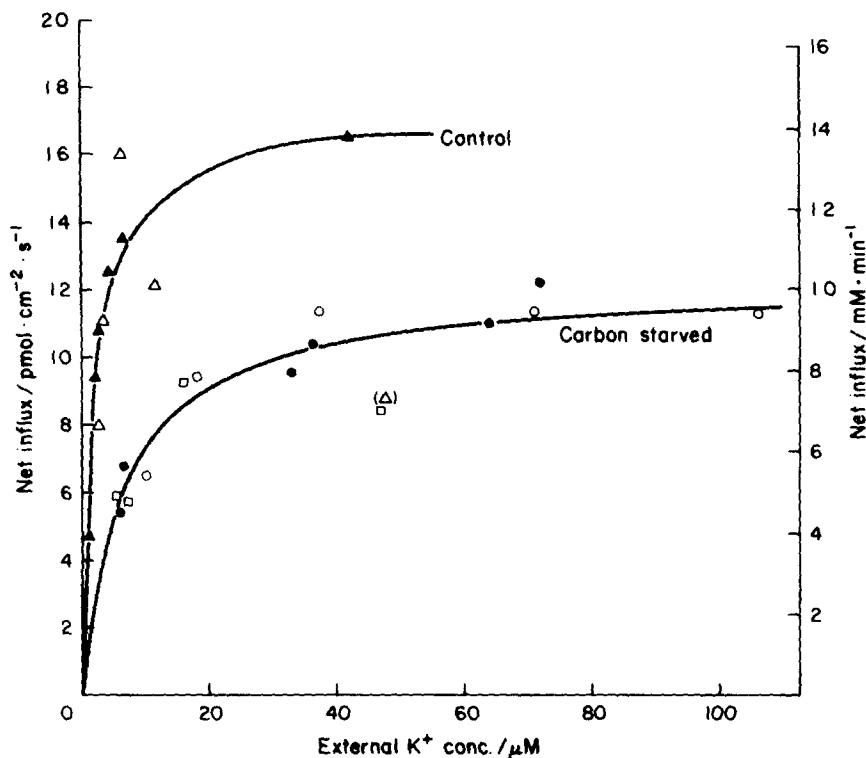

FIGURE 2. Concentration dependence of K^+ uptake in low-K^+ shaking-culture cells. K^+ flux was measured as the initial slope of disappearance from the medium, during the first minute after addition of K_2SO_4 (samples at ~15, 30, 60, and 90 s). Each symbol represents the average of duplicate determinations. Circles indicate net uptake; squares and triangles represent $^{42}K^+$ uptake. Control cells (triangles) were preincubated in buffer with 1% glucose for 15–45 min before ^{42}K-labeled potassium was injected. Carbon-starved cells (circles and squares) were prepared by 3 h incubation in buffer without added sugar. Growth medium: AmVM; flux buffer: CaMES. The curves drawn are Michaelis curves fitted by least squares (Marquardt, 1963): $J_{max} = 14.4 \pm 1.0$ mM/min and $K_{1/2} = 2.0 \pm 0.5$ μM, for the control cells (with glucose); $J_{max} = 10.1 \pm 0.4$ mM/min and $K_{1/2} = 6.7 \pm 1.0$ μM, for the carbon-starved cells. The single triangle in parentheses was omitted from fitting of the control data.

pH₀ Sensitivity of High-Affinity K⁺ Transport

Net potassium uptake by starved mycelium maintained without added calcium was previously found to be balanced ~65% by extrusion of Na ions and 35% by extrusion of H ions (Slayman and Slayman, 1968), giving an approximate ion stoichiometry ($K^+:Na^+:H^+$) of 3:2:1 at the control pH of 5.8. Saturating potassium

efflux showed a weak dependence on extracellular pH, declining by only 50% from the optimum (~20 mM/min), at pH 5, to a plateau spanning the range pH 7 to pH 8.5. Sodium flux, however, had a much stronger pH dependence, declining by 90% from pH 5 to pH 8, so that at high pH the ion stoichiometry was essentially 1 K^+:1 H^+ (Slayman and Slayman, 1970).
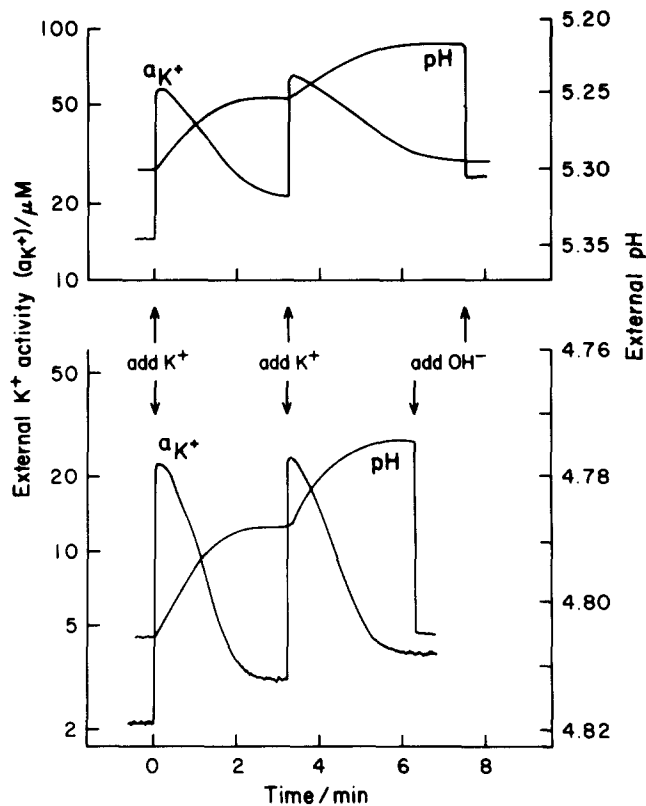

FIGURE 3.   Demonstration of net H^+ efflux accompanying high-affinity K^+ uptake. Spherical cells (upper panel) and shaking-culture cells (lower panel) were suspended in 20 ml of CaBES buffer, within a thermostatted, water-jacketed vessel. Extracellular pH and K^+ were measured with ion-specific electrodes. Sudden vertical shifts indicate additions of K_2SO_4 (750 nmol K^+ in the upper panel, 500 nmol in the lower panel) or NaOH (1,500 nmol OH^- in the upper panel, 1,000 nmol in the lower panel) and serve as calibrations. Time courses of K^+ uptake and H^+ release by the cells are given by the slow shifts immediately after K^+ addition. K^+/H^+ exchange ratio: 0.95 referred to rates and 0.84 referred to the total exchange, for the upper panel; 0.94 referred to rates and 0.90 referred to total exchange, for the lower panel.

However, sodium is not required for the growth or maintenance of *Neurospora* (Slayman and Tatum, 1964), and since it can interfere with potassium uptake, it was eliminated from most of the present experiments by omission from the growth medium (AmVM) and test buffer (CaMES). Not surprisingly, then, net potassium uptake via the high-affinity mechanism was entirely balanced by net

extrusion of protons. In Fig. 3, simultaneous traces from extracellular pH and K^+ electrodes demonstrate the coincident uptake of potassium and release of protons that occurred when low-K^+ cells were suddenly given potassium. In five experiments with shaking-culture cells and three with spherical cells, similar to the runs shown in Fig. 3, K^+ influxes and H^+ effluxes matched, with an average ratio (K^+/H^+) of 0.96 ± 0.05. The dependence of net K^+ influx upon pH_o showed a clear optimum near the normal pH of 5.8; the saturating flux declined to 30% of that value at pH 8 (Fig. 4).
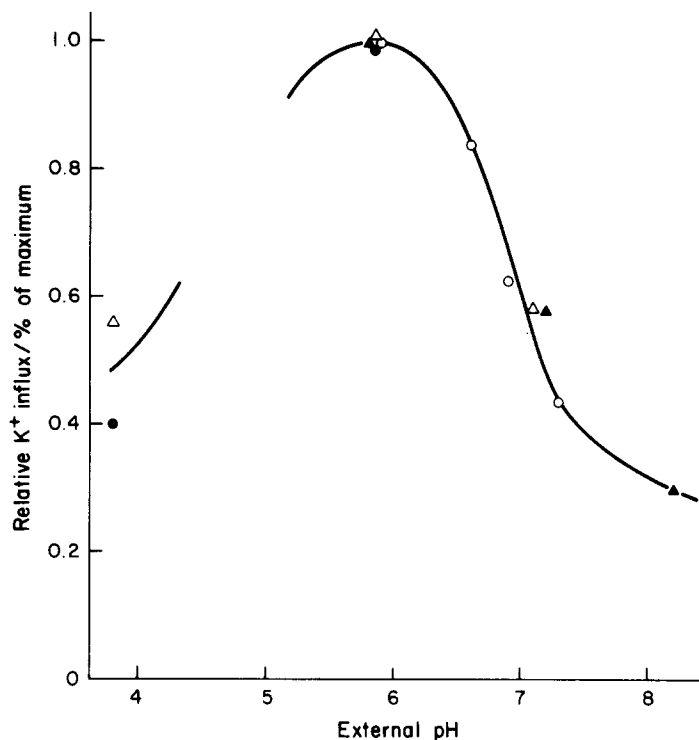

FIGURE 4. pH optimum of high-affinity potassium uptake by *Neurospora*. Low-K^+ spherical cells were maintained in CaMES buffer. The setup and flux calculation are the same as in Fig. 2. Solid symbols indicate $^{42}K^+$ flux; open symbols indicate net flux. Each symbol represents the average for duplicate measurements, and the different symbols designate separate experiments.

Effects upon Membrane Potential

Although previous measurements (Slayman, 1970) had indicated that net K^+ flux into mature *Neurospora* mycelium had little or no effect on membrane potentials, cytoplasmic depletion of potassium was not well-controlled in those studies. Electrical experiments on K^+-starved spherical cells have given quite a different result: micromolar K^+ can cause large depolarizations, which occur from surprisingly high resting membrane potentials. The average resting potential (V_m) of normal spherical cells, when bathed in standard dimethyl glutarate

(StDMG; containing 25 mM K^+ and 1 mM Ca^{++}; see Table I) is -200 ± 8 mV (cell interior negative), and the resting membrane resistivity (R_m) is 64 ± 6 kΩ· cm^2 (Blatt and Slayman, 1983). Survey measurements in the present experiments gave corresponding figures of -218 ± 7 mV and 65 ± 8 kΩ·cm^2 for normal-K^+ cells bathed in CaMES (0 added K^+; 0.6 μM, actual). However, low-K^+ spherical cells in the same medium displayed V_m values of -299 ± 3 mV, with an observed maximal value of -367 mV. These measurements were made over a period of 5 mo, on 35 independent cells. R_m values for the same cells averaged 57 ± 4 kΩ· cm^2, not significantly different from the values for normal-K^+ cells.
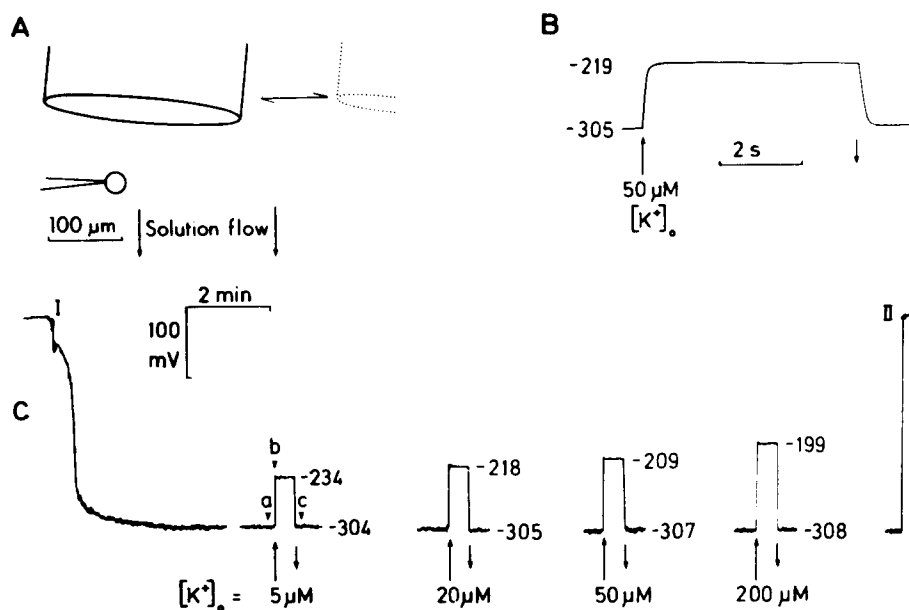

FIGURE 5.   Effect of added extracellular potassium on membrane potential in low-K^+ spherical cells. (A) Diagram of the arrangement of the cell, the impaling electrode, and a K^+-flooding pipette for rapid addition and removal of potassium (see Materials and Methods). (B) Depolarization with 50 μM K^+ on an expanded time scale, to show rapidity of the voltage shift. (C) Condensed record from a single cell, showing voltage responses to four different K^+ concentrations. The cell was impaled at I, and the seal was formed over a period of 45 s, to give a resting membrane potential near -300 mV (cell interior negative). The inverted triangles indicate typical times when I-V scans were run (see Fig. 6). The impaling electrode was removed at II. These are typical records, from a cell bathed in CaMES.

The addition of micromolar K^+ to the medium bathing potassium-starved cells resulted in large, rapid, sustained depolarizing (positive) shifts of membrane potential, as is illustrated in Fig. 5. Washout of the added potassium gave complete recovery, even after 10–20-min exposures. (Longer exposures, 50–60 min, caused partially irreversible depolarization.) Voltage responses to addition or removal of K^+ occurred with time constants of a few tens of milliseconds (see expanded-scale record of Fig. 5B), which is consistent with bulk solution mixing and with the cell membrane time constant. Observed depolarizations with satu-

rating K^+ (i.e., 50 μM) spread between 25 and 150 mV and had a mean value of 78 ± 4 mV in 35 observations. The corresponding decrease in membrane resistivity was 25 ± 3 kΩ·cm^2. These voltage and resistance data are summarized in Table II.

Qualitatively, all of the data thus far presented—including potassium influx, proton extrusion, and membrane depolarization—could be accounted for by a high-affinity potassium carrier or channel (potassium uniport), through which ions are driven inward by the membrane potential, which is itself in turn established by the primary proton pump of the *Neurospora* plasma membrane. Given a stable V_m of −300 mV, for example, cytoplasmic K^+ at 100 mM could theoretically be sustained against an extracellular $[K^+]$ of 1 μM. However, the question of whether such a simple mechanism actually operates cannot be resolved without a careful quantitative comparison of the voltages, currents, and potassium concentrations involved.

TABLE II

Membrane Potentials and Resistances in Low-K^+ Spherical Cells

	0 K^+	50 μM K^+	Difference
Membrane potential (mV)	−299±3	−221±4	78±4
Membrane resistance (kΩ·cm^2)	57±4	32±2	25±3

Measurements were made with double-barreled microelectrodes, as described in Materials and Methods. CaMES buffer with added K_2SO_4 was used. Values are means ± 1 SEM for 35 cells.

K^+ Activity, E_K, and the Need for Ion Coupling

As a first (thermodynamic) check on possible mechanisms for K^+ uptake, membrane potentials and cytoplasmic ion activities were determined in spherical cells under a variety of conditions. Table III summarizes the results from five experiments: three with cells grown in the ammonium phosphate medium (AmVM, Table I), and two with cells grown in Na phosphate medium (NaVM). In the four experiments carried out with potassium acetate-filled pipettes (cells 1–4), a_{Ki} averaged 54 ± 3 mM and showed no consistent change with time before or after K^+ was added to the medium. Evidently cytoplasmic $[K^+]$ is effectively clamped by the pipette-filling solution. For cells maintained at the control pH_o (5.8), resting membrane potentials averaged −286 ± 7 mV in the absence of added potassium (column 9), with 50–180 mV depolarization upon addition of 50 μM $[K^+]_o$. Membrane potentials for each cell after addition of K^+ (20–200 μM) are listed in column 10. To test the operation of a K^+ uniport mechanism, these numbers must be compared with the equilibrium diffusion potentials for that ion (E_K), shown in column 11 of Table III. Clearly, in most cases, E_K in the presence of 50 μM K^+ was well negative to the cell membrane potentials, and it was possible in the other cases (cells 4 and 5) to achieve that condition by lowering extracellular pH (column 7). Thus, simple uniport mechanisms, in which potassium would be driven inward by the membrane potential, cannot account for the observed K^+ accumulation.

On the other hand, the diffusion potentials for protons (E_H; column 12) were generally far positive to the membrane potential during K^+ uptake, and this fact,

along with knowledge that many other substrate accumulation processes in *Neurospora* are coupled to influx of protons (Slayman, 1974; Eddy, 1982), raised the question of whether the high-affinity K^+ system might also operate via a proton cotransport mechanism. Assuming, for the sake of argument, that no phosphate-bond or redox energy is involved, the combined equilibrium potential for coupled movement of n K ions with m H ions can be obtained from the mass reaction equation, using electrochemical activities ($\tilde{a}_{Ki}$, etc.), instead of concen-

TABLE III

Ion Gradients and Transport Potentials in Low-K^+ Spherical Cells

1	2	3	4	5	6	7	8	9 Membrane potential	10	11	12	13	14
Cell	Time	Electrode salt	Growth medium	Added K_o^+	a_{Ki}	pH_o	pH_i	$-K^+$	$+K^+$	E_K	E_H	E_{KH}	I_K
	min	*mM*		*μM*	*mM*			*mV*	*mV*	*mV*	*mV*	*mV*	*peq·cm^{-2}·s^{-1}*
1	9	50 KOAc	AmVM	50	55	5.8	7.02	−285	−143	−178	+71	−54	17.3
2	28	50 KOAc	AmVM	50	55	5.8	7.02	−305	−145	−178	+71	−54	16.5
					57			−295	−120	−179		−54	19.5
					58			−298	−119	−180		−54	20.6
3	6	50 NaVM	NaVM	200	45	5.8	7.02	−290	−169	−137	+71	−33	26.3
	17	KOAc		20	46			−290	−195	−194		−62	15.6
4	18	50 NaVM	NaVM	50	66	5.8	7.02	−275	−220	−182	+71	−55	16.7
	24	KOAc			49	3.9	6.87	−115	−89	−175	+174	0	11.2
5	9	100 NaOAc	AmVM	50	10	5.8	7.02	−250	−200	−134	+71	−32	14.6
	28				5	7.2	7.13	−280	−205	−117	−4	−61	8.4
	45				3	3.9	6.87	−74	−103	−104	+174	+35	6.3

Single-ion diffusion potentials (E_K, E_H) were calculated by the Nernst equation, using the measured values of a_{Ki} (column 6), measured pH_o (column 7), $[K^+]_o$ estimated from parallel experiments (22, 51, and 205 μM; assumed activity coefficient of unity), and pH_i (column 8) measured in parallel experiments. Equilibrium potentials for cotransport (E_{KH}) were calculated from text Eq. 4, using the same data and a stoichiometry of 1 K^+:1 H^+ ($= n = m$). The time of measurement after each impalement is given in column 2; impalements generally lasted about twice the maximal time shown. CaMES buffer with potassium added as KCl was used; background K^+ before addition was 0.2–0.7 μM.

trations, for each ion. The reaction is simply

$$n \, K_o^+ + m \, H_o^+ \rightleftharpoons n \, K_i^+ + m \, H_i^+, \tag{1}$$
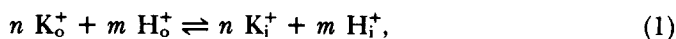
for which the mass reaction equation is

$$K = 1 = \left(\frac{\tilde{a}_{Ko}}{\tilde{a}_{Ki}}\right)^n \cdot \left(\frac{\tilde{a}_{Ho}}{\tilde{a}_{Hi}}\right)^m. \tag{2}$$

Electrochemical activity is defined by

$$\tilde{a}_{Ki} = a_{Ki} \cdot \exp(zFV_i/RT), \tag{3}$$

in which a is the chemical activity; R, T, and F have their usual meanings; z (valence) $= 1$; and the subscripts i and o designate cytoplasm and extracellular solution, respectively. Combining Eq. 3 with Eq. 2 and rearranging gives the

membrane potential when the putative cotransport system is at equilibrium:

$$V_m = (V_i - V_o) = \frac{1}{n+m} \cdot \frac{RT}{F} \left[n \cdot \ln\left(\frac{a_{Ko}}{a_{Ki}}\right) + m \cdot \ln\left(\frac{a_{Ho}}{a_{Hi}}\right) \right]. \tag{4}$$

The transport reversal potential thus calculated, which is designated E_{KH} in Table III (column 13), depends upon the assumed values of n and m, and upon the assumed activity coefficient (γ_{Ko}) for extracellular potassium. Letting $n = m = 1$ and $\gamma_{Ko} \simeq$ unity in solutions of 6 mM ionic strength (CaMES; see Robinson and Stokes, 1959) gives the values in Table III. (In fact, when $n = m = 1$, E_{KH} must lie halfway between E_K and E_H.) A comparison with column 8 shows that in all cases E_{KH} is positive to the actual membrane potentials measured during potassium uptake, so that electrophoretic K^+-H^+ cotransport is at least thermodynamically feasible.

Charge Stoichiometry of Potassium Uptake

Taken at face value, the K^+/H^+ exchange behavior observed (Fig. 3) during high-affinity potassium uptake would seem to argue against a K^+-H^+ cotransport mechanism. However, since in *Neurospora* the return pathway for most noncapacitative current carried inward with potassium is the primary proton extrusion pump (Slayman and Gradmann, 1975), the apparent K^+/H^+ exchange could well be generated physically by separate movements of K^+-H^+ and 2 H^+. The crucial question, then, is whether protons do in fact enter stoichiometrically with potassium, via the high-affinity system.

The most elegant way to answer this question would be to use ion-sensitive microelectrodes to measure changes in both cytoplasmic pH and cytoplasmic $[K^+]$, immediately after potassium is given to depleted cells. However, the concentration-clamping effect of solutions in the impaling reference electrodes vitiates such an approach for K^+ (cf. columns 3 and 6 of Table III) in these small spherical cells. It was therefore necessary to compare the electric current, measured on single cells, with net K^+ accumulation measured on populations of (unimpaled) cells. Steady state current-voltage (I-V) curves for the *Neurospora* membrane were determined by means of a computer-driven voltage-clamp arrangement (see Materials and Methods) similar to that described previously (Gradmann et al., 1978), but using only a single electrode barrel to measure voltage (as allowed by the spherical geometry of these cells). Fig. 6 illustrates the I-V plots obtained immediately before the potassium (50 μM) stream was added, in the presence of K^+, and immediately after the stream was removed. If it is assumed that the only effect of potassium on the cells was to supply substrate for the K^+ uptake system, then current through that system under non–voltage-clamped conditions must be equal to the vertical separation between the control curves and the test curve, at the stable V_m achieved during K^+ uptake. A summary of such currents, obtained at four different K^+ concentrations, is given in Table IV (column 2). The corresponding summary of net potassium influxes is given in column 3 of the table. Those fluxes appeared to be proportional to the currents, with the ratios (I_K/J_K) shown in column 5.

Fig. 7 provides a graphic display of complete data from three flux experiments and two current experiments. The curves drawn through these data are rectan-
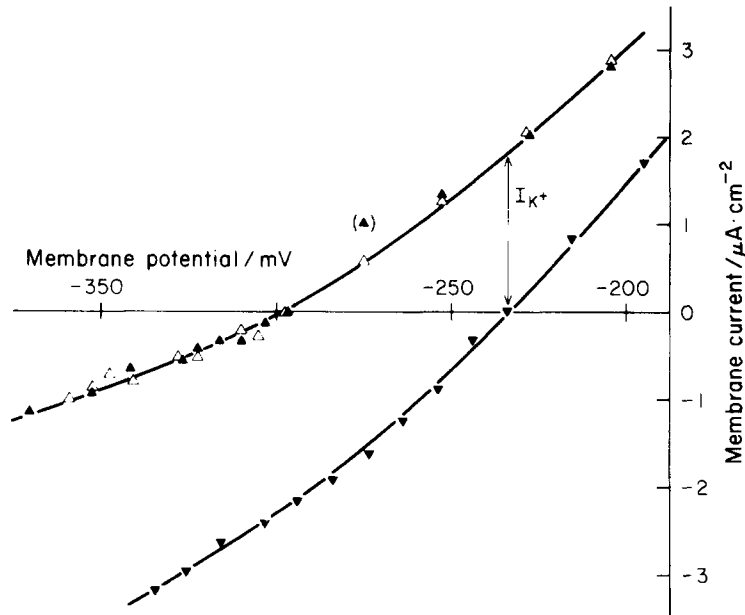
FIGURE 6.   Effect of added potassium on the membrane current-voltage curves for low-K spherical cells. This experiment was similar to that in Fig. 5, with I-V scans taken at the times designated, relative to addition of 50 μM KCl. Before addition (▲), in the presence of KCl (▼), and after removal of (△). Current through the K^+ transport system is calculated as the difference current between the control I-V curve (upper curve, average of the two plots), and that obtained in the presence of K^+ (lower curve), at the membrane potential prevailing in the presence of potassium. Smooth curves were fitted by least squares (Marquardt, 1963) to second-degree polynomials: $y = a_0 + a_1x + a_2x^2$; coefficients are 14.8, 0.077, and 0.000092, respectively, for the control curve, and 14.7, 0.085, and 0.000094, for the test curve.

gular hyperbolas (Michaelis curves) fitted by least squares, with a common value for $K_{1/2}$ and separate values of maximal velocity: $K_{1/2} = 14.9 \pm 2.6$ μM, $I_{max} = 30.1 \pm 1.6$ pmol·cm^{-2}·s^{-1} for current, and $J_{max} = 15.3 \pm 1.0$ pmol·cm^{-2}·s^{-1} for net K^+ flux. It is evident, from both Table IV and Fig. 8, that two electric charges

TABLE IV

Current Stoichiometry for High-Affinity K^+ Uptake in Neurospora

(K_o^+)	I_K	K^+ uptake	I_K/K^+ uptake
μM	$peq \cdot cm^{-2}s^{-1}$	$pmol \cdot cm^{-2}s^{-1}$	$eq \cdot mol^{-1}$
6.7	12.3	5.8	2.1
21.9	16.3	8.9	1.9
50.5	20.6±0.6	9.2±0.4	2.2±0.3
205	29.1	16.3	1.8

Low-K^+ spherical cells were used; net influx of K^+ was measured. Associated transport currents were measured as described in Fig. 7. Except at $[K^+]_o \simeq 50$ μM, the values listed are the means of two or three separate measurements (having maximal spreads of $\leq 10\%$). At 50 μM K^+, there were 28 current experiments and 11 flux experiments.

entered the cell for every K ion. Since only one proton could be seen exiting the cells, via the proton pump (Fig. 3), the extra charge entering with K^+ must have been H^+.

An examination of the plots in Figs. 2 and 7, along with the records in Fig. 5, seems to reveal a systematic difference between spherical cells of *Neurospora* and shaking-culture cells. For spherical cells, the voltage steps (Fig. 5), as well as the transport currents and fluxes (Fig. 7), are unexpectedly large at the very low
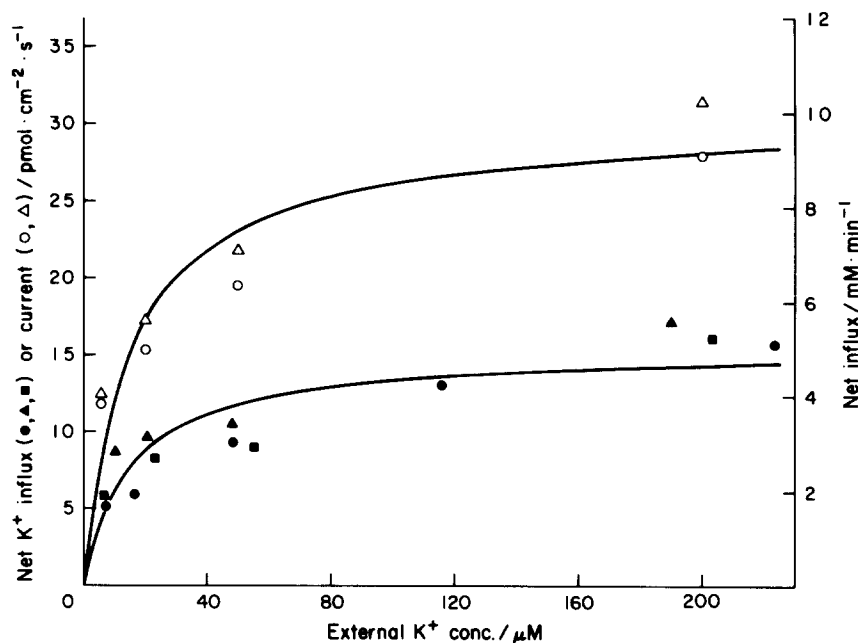

FIGURE 7. Stoichiometry of the K^+-H^+ symport, from parallel measurements of transport current and net K^+ flux. Upper plot: currents, determined as in Fig. 6, in response to different added K^+ concentrations. Two experiments. Lower plot: net fluxes, determined as described for Fig. 2, in three separate experiments on spherical cells. The smooth curves are Michaelis functions fitted jointly to the two sets of data, using a common value for $K_{1/2}$ of 14.9 μM. The separate values of J_{max} are 15.3 ± 1.0 pmol·cm^{-1}·s^{-1} for the flux, and 30.1 ± 1.6 pmol·cm^{-2}·s^{-1} for the current. The stoichiometric ratio current/net K^+ flux, whether calculated from the observed fluxes and currents at corresponding concentrations (i.e., Table IV) or from the fitted curves, is very close to 2.

K_o^+ concentrations (i.e., 10 μM and lower); they also appear somewhat small, compared with the Michaelis fits, in the midrange of concentrations (~50 μM). These two displacements have the effect of raising the calculated K_m for K^+ transport by the spherical cells (cf. 14.9 μM in Fig. 7 with 6.7 μM in Fig. 2) and giving the appearance of two flux components. However, the available data do not support this interpretation on statistical grounds, so its existence and possible cause must be left open for the time being. Fortunately, these questions do not affect the basic argument about charge stoichiometry.

Considerations of Unidirectional Flux

One caveat in the determination of stoichiometry is that if a significant K^+ efflux were to occur during the observed net influx, then the actual unidirectional K^+ flux through the high-affinity transport system would be larger than indicated in Table IV or Fig. 7, relative to the charge transport. In that case, the required fraction of coupled proton influx would be reduced, conceivably to zero. This is an unattractive possibility, for teleological reasons: it would be economically
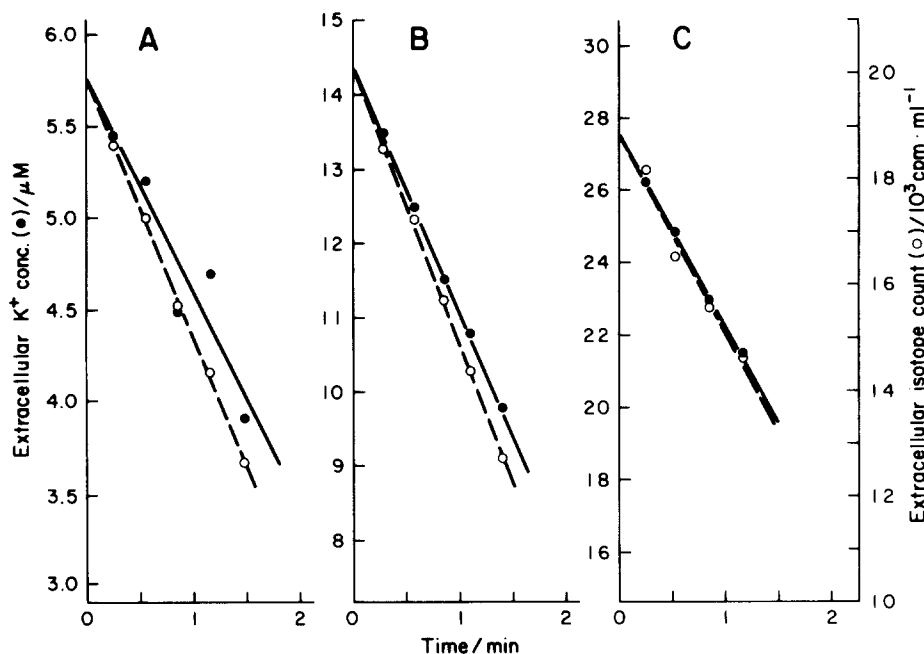

FIGURE 8. Comparison of unidirectional and net fluxes during high-affinity uptake of K^+. Simultaneous removal of $^{42}K^+$ and chemical K^+ from the suspending medium, as a function of time. Shaking-culture cells were grown in AmVM and incubated in a pH 7 buffer (1 mM HEPES titrated with NaOH, 1% glucose, and free calcium and magnesium levels as in AmVM), before addition of K_2SO_4. Initial $[K^+] = 5.7$ (A), 14.3 (B), and 27.5 (C) μM. Solid circles indicate net flux; open circles indicate $^{42}K^+$ flux. The three left-hand ordinates are scaled, relative to the right-hand ordinate, according to the specific activity of isotope in the three suspensions.

foolish of K^+-starved cells to release one K ion for every two taken up, and it would make inefficient use of the driving force represented by the membrane potential. Obviously, however, careful unidirectional flux measurements are required to answer the question.

For that purpose, several experiments were carried out to determine isotopic $^{42}K^+$ uptake and net K^+ uptake simultaneously on the same cells. Similar results were obtained in three experiments on spherical cells and two experiments on shaking-culture cells, and one of the latter experiments is shown in Fig. 8. In all cases, the disappearance of $^{42}K^+$ into the cells was faster than the disappearance

of chemical potassium, the ratio of slopes being 1.21, 1.12, and 1.04 in the examples of Fig. 8, and averaging 1.20 ± 0.03 for all experiments. The slope ratios varied randomly, independent of K^+ concentration, of extracellular pH (7.0 and 5.8 tested), and of carbon starvation. It is possible, therefore, that 20% of the excess current shown in Table IV could be carried by entering K ions. That is not sufficient to negate the basic notion of electrophoretic K^+-H^+ cotransport.

DISCUSSION

The operation of a potassium-proton symport as the primary mechanism for high-affinity uptake of K^+ represents a new departure in the understanding of cation sequestering in fungi and plants. Three major facts support the idea for *Neurospora*. (*a*) Current measurements show that two charges enter the cells for each potassium ion (Figs. 6 and 7). (*b*) Net proton efflux occurs during potassium uptake, with an overall stoichiometry of 1:1. Because the major electric source to drive potassium transport is the proton extrusion pump in the *Neurospora* plasma membrane, items *a* and *b* above require H^+ to be the second charge entering with K^+. (*c*) Finally, a symport mechanism is thermodynamically competent to support the maximal potassium gradients observed in *Neurospora* during transport depolarization (Table III), whereas uniport mechanisms (carriers or channels) and electroneutral ion exchangers are not thermodynamically competent. The latter observation has been used previously to argue for K^+-H^+ symports in yeast (Boxman et al., 1984) and in bacteria (Bakker and Harold, 1980). Although the participation of an ATP-fueled, inwardly directed K^+ pump cannot be rigorously excluded by our data, the operation of such a device under the above constraints would be unreasonably inefficient: it would require net hydrolysis of three ATP molecules to accomplish the entry of a single K ion, with two of the ATP molecules consumed solely to cycle protons. By contrast, a K^+-H^+ symport driven by the membrane potential consumes two ATP molecules for each K ion taken up, one for each charge extruded by the proton pump. Although one of these ATP molecules is also used to cycle protons, it has the explicit purpose of bolstering the potassium transport system against other energy losses in the membrane. Finally, it should be added that purification of membrane-bound ATPases in *Neurospora*, carried out by several different laboratories on several different membrane fractions, has revealed no potassium-specific ATPase.

Any K^+-H^+ symport mechanism, which by definition requires that extracellular protons be bound to the "carrier" and transported, could be expected to be strongly sensitive to extracellular pH, at least from an intuitive point of view. Experiments on this point will be detailed in another paper (Blatt, M. R., C. L. Slayman, and A. Rodriguez-Navarro, manuscript in preparation), but can be summarized as follows: variation of extracellular pH from 8.2 down to 5.2 enhances the K^+-associated inward current about fivefold (at saturating voltages; −320 mV), along a quasi-titration curve with an apparent pK near pH 7. In this case, again, the current data closely resemble the K^+ flux data (Fig. 4).

Purely from the point of view of comparative physiology, the possible operation of potassium-proton symport mechanisms should be studied among the other

familiar ascomycetes (e.g., *Saccharomyces* and *Neocosmospora*) and deuteromycetes (e.g., *Aspergillus, Candida, Torulopsis,* and *Rhodotorula*). A much larger potential significance, however, lies in the extensive similarities between cation transport phenomena observed in fungi and those observed in algae and higher plants (reviewed in the Introduction), or more generally among all organisms whose overall transport economy is based on primary proton pumping. The following discussion outlines how the existence of predominant K^+-H^+ cotransport could be reconciled with a variety of established observations on K^+ transport in plants and fungi.

Measured Stoichiometric K^+/H^+ Exchange

The way in which the operation of a K^+-H^+ symport could be masked by coordinated functioning of the primary proton pump has already been discussed in the Results (*Charge Stoichiometry*). However, as has been found for the glycine-proton symport in *Saccharomyces* (Ballarin-Denti et al., 1984) and for the glucose-proton symport in *Neurospora* (Sanders and Slayman, 1984), compensatory pumped efflux of protons can occur without a reliably detectable fall of intracellular pH. Therefore, reported failures to find a change of intracellular pH during potassium uptake (Roberts et al., 1982) cannot themselves be taken as evidence against K^+-H^+ cotransport. Detailed arguments about ionic compensation vary, depending on the properties of the proton pump, but the essential point is that under steady state conditions, charge influx through the symport must be neutralized by charge efflux elsewhere, either through the pump or through leakage pathways. When the fraction of charge compensation caused by protons nears 100%, then net proton efflux must approximate the net K^+ influx.

Inhibition of K^+ Transport

It is a common observation that potassium transport in plants and fungi is blocked by metabolic inhibitors that either deplete the cells' energy supply or specifically attack the primary proton pump. Although a strictly H^+-coupled transport system—like the high-affinity potassium, glucose, or amino acid uptake systems in *Neurospora*—might be imagined, on a priori grounds, to be independent of energy metabolism or immediate proton pumping, there are several mechanisms that could account for the observed inhibitor effects. A trivial mechanism would be nonspecific targeting of the inhibitor. Thus, blockade of K^+ transport in corn roots (Cheeseman et al., 1980) by the ATPase inhibitor dicyclohexylcarbodiimide (DCCD) could come about by direct action on the K^+-H^+ cotransport system, if DCCD binds to proton "channel" elements, as it is believed to do in bacteriorhodopsin (Renthal et al., 1981), in the F_o moiety of the mitochondrial and bacterial H^+-ATPases (Fillingame, 1980), and in the fungal proton ATPase (Sussman and Slayman, 1983).

An indirect but more interesting mechanism for inhibitor action on K^+-H^+ cotransport systems is a kinetic limitation produced rapidly by an alteration of proton pumping. Cyanide blockade of *Neurospora*, for example, causes membrane depolarization within 10–30 s (Slayman et al., 1973) and cytoplasmic acidification (from pH 7.2 to ~6.6) within a few minutes (Sanders and Slayman, 1982). Transport systems that are either strongly voltage dependent (like the

K^+-H^+ symport in *Neurospora*; Blatt, M. R., A. Rodriguez-Navarro, and C. L. Slayman, manuscript in preparation) or strongly pH_i dependent (like the glucose-H^+ mechanism in *Neurospora*) would be vulnerable to such inhibition, but should simultaneously display clear kinetic changes, as has been observed, for example, for potassium transport in corn roots (Cheeseman et al., 1980).

Finally, it is clear that some cotransport systems, which might include K^+-H^+ systems, can be inhibited by rapid-acting but more remote mechanisms. The glucose-proton symport of *Neurospora*, for example, is stopped within 15 s by millimolar cyanide (Slayman, 1977, 1980), whose direct action is to block respiration at cytochrome a-a_3 and thereby cause ATP to fall, but over a period of 30 s. Unlike the K^+-H^+ symport, however, the glucose-H^+ symport behaves like a current source (Hansen and Slayman, 1977), being insensitive to the membrane voltage per se. Since the cyanide-induced drop of cytoplasmic pH takes place over several minutes, the only device for promptly stopping the glucose carrier would have to be a messenger-mediated controller with an acute sensor.

Dual-Range Saturation Kinetics

Finally, K^+-H^+ cotransport is capable of accounting for one of the long-standing observational disputes in the literature of plant physiology: dual-range saturation kinetics for cation transport (Epstein, 1966; Nissen, 1973). In the operation of cotransport systems, it is often imagined (for simplicity) that only the doubly complexed form of the carrier can cross the membrane with the transported substrate. In many experimental cases, that seems to be a good approximation. However, if the singly complexed form of the carrier (i.e., K^+ bound without H^+) can also occasionally cross, then dual-range saturation kinetics will appear when the K^+ concentration becomes high enough to tie up an appreciable fraction of the carrier in H^+-free states (Sanders and Slayman, 1983; Sanders, 1986). Such an effect should be accentuated at high pH, and extreme extracellular pH's ought to be tested on the dual-range mechanisms already described. In addition, of course, the voltage dependence of the K^+-H^+ symport in *Neurospora* leaves open the possibility that dual-range kinetics may arise because high $[K^+]_o$ causes depolarization, as suggested more than 10 years ago by Gerson and Poole (1971).

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