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Polyamine Transport in *Neurospora crassa*[1]

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Polyamine transport in *Neurospora crassa* is concentrative and energy dependent in a dilute buffer. The saturable systems governing the uptake of putrescine (K_m = 0.6 mM), spermidine (K_m = ca. 0.24 mM), and spermine (K_m = 0.07 mM) share components, as indicated by mutual inhibition among the polyamines. In addition, nonsaturable components prevail for putrescine and spermidine, particularly the former. Radiolabeled substrates, once in the cell, are released only slowly, even if unlabeled polyamines are included in the incubation medium. Permeabilization of cells with *n*-butanol leads to partial release of internalized ^{14}C-polyamines, and the remainder is almost wholly exchangeable with added, unlabeled polyamine. Polyamine uptake was inhibited by the polyamines themselves and by a polyamine analog, methylglyoxal bisguanylhydrazone, but only weakly and incompletely by the basic amino acids arginine and ornithine. Uptake of putrescine and spermidine was inhibited by monovalent cations, Ca^{2+}, and certain other components of the growth medium. As a result, uptake from the growth medium was very slow and largely by way of the nonsaturable uptake mechanism.

The metabolism of polyamines (putrescine, spermidine, and spermine) has become an active research area, owing to the close connection between polyamine synthesis and growth in most organisms, although it is not fully understood what cellular functions polyamines serve. The regulatory mechanisms of the two initial enzymes of the pathway, ornithine decarboxylase and *S*-adenosylmethionine decarboxylase, are varied and unusual (1-4). The many studies of polyamine-mediated enzyme regulation are complicated by the polycationic nature of the polyamines, which leads them to bind to anionic constituents of the cell (5, 6), and their consequent metabolic compartmentation (7). Moreover, the polyamines are toxic to some cell types, and close control of polyamine uptake, synthesis, turnover, and cellular distribution is all the more important.

The filamentous fungus, *Neurospora crassa*, is a simple eucaryote in which polyamine metabolism and regulation resemble those of the mammalian pathway (8, 9). Like investigators of other organisms, we have shown that the polyamines themselves regulate the rate of polyamine synthesis, not by feedback inhibition, but in part by effects on ornithine decarboxylase synthesis and degradation (9, 10). We have shown that the diffusible, regulatory pools of these compounds are a small portion of the total cellular polyamines (7), and that substantial regulatory effects might follow changes in the internal distribution of the polyamines (8). We have found that addition of polyamines to cells affects the pathway only sluggishly, largely because polyamine uptake from growth medium is slow

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(8). In order to understand the uptake process, to gain control of introducing polyamines into cells, and to select mutants for polyamine uptake, resistance, and regulation, we have studied the basic features of polyamine transport.

MATERIALS AND METHODS

Strains and growth medium. The wild-type strain used was *N. crassa* strain ORS-6a. Strain IC1984-53a carried an *aga* mutation (arginase-deficient, allele UM-906) and a *spe-1* mutation (ornithine decarboxylase-deficient, allele LV10) (11). Strain IC2448-54A carried the LV10 mutation. The growth medium was Vogel's medium N (12) with 1.5% sucrose, and supplemented with 1 mM spermidine if necessary for growth of Spe^- strains. For reference, the concentrations of the major cations of Vogel's medium are 24 mM Na^+, 37 mM K^+, 25 mM NH_4^+, 0.8 mM Ca^{2+}, and 0.7 mM Mg^{2+}. (The citrate present in this medium, by chelation, lowers the free divalent cation levels to as little as one-tenth their nominal concentrations.) Starvation for K^+ was done by replacing all K^+ of the normal medium with the corresponding Na^+ salt, and adding KCl to the desired concentration (13).

Mycelia used to measure uptake rates were grown in 400- to 1000-ml exponential cultures, inoculated with about 1×10^6 conidia/ml. The cultures were contained in boiling flasks, connected to a hydrated source of air through a tube held by the cotton plug and thrust to the bottom of the flask. The flasks were held in a water bath at 25°C. Dry weights were monitored by filtering 20-ml lots of culture and drying the mycelia with acetone (14).

Measurement of uptake rates. Cells were grown in aerated, exponential cultures to 0.5–0.6 mg dry weight/ml (about 10–12 h after inoculation). They were harvested and washed with Na^+–Mops[3] buffer (20 mM Na^+, pH 7.2) with 0.2% glucose, and resuspended in their original volume in the same buffer. They were equilibrated for 30 min at 25°C in an Erlenmeyer flask with rotary shaking. Uptake rates were determined by transferring 9 ml of mycelial suspension to 50-ml plastic tubes (Falcon No. 2070), held at 25°C with shaking, and containing ^{14}C-polyamine substrate. Zero-time samples (2 ml, in duplicate) were taken; cells were collected on prewashed 5-μm membrane filters and washed with 0.25 M NaCl to remove external radioactivity. The filters were collected, placed in scintillation counting vials in 0.5 ml 0.4 M $HClO_4$ + 2 mM EDTA for 30 min, and counted after adding scintillation fluid. The preincubation time regularized uptake behavior, but cells tested immediately after being washed behaved similarly.

Under standard conditions, cells grown in minimal medium took up putrescine in a linear fashion for at least 10 min. Spermidine uptake was linear with time for at least 15 min. The "age" of the culture was standardized at 0.5–0.7 mg dry weight at the time of use, owing to the increasing uptake capacity during growth (see Results). Similarly, the Na^+ concentration was standardized at 20 mM, because of the sensitivity of uptake to cation.

Inhibitors were added either during the beginning of the equilibration step or at the time the radioactive substrate was added, as noted under Results. In some cases, uptake from the growth medium was tested.

Metabolite pools. HPLC analysis of polyamine pools was done as previously described (8). The fate of ^{14}C-polyamines was studied after $HClO_4$ extraction of cells exposed to substrate. The acid-insoluble material was counted, and the extract was fractionated on AG-50W-X8 cation-exchange columns (Na^+ form, 200–400 mesh) as described previously (15). Eluate fractions were monitored for radioactivity. The most basic fractions, emerging in 0.2 N NaOH, were desalted on AG-50 columns in the H^+ form and eluted with 6 N HCl. The evaporated samples were analyzed by HPLC for additional nonpolyamine products. The raw $HClO_4$ extracts were also dansylated and analyzed for acetylpolyamines by thin-layer chromatography (16).

Materials. All biochemicals were from Sigma Chemical Co. (St. Louis, MO). Isotopically labeled compounds were from Amersham or New England Nuclear Corp.

RESULTS

Polyamine Concentration

The concentration dependence of polyamine uptake suggested at least two kinetic components for both putrescine and spermidine. The raw data for putrescine could be resolved into saturable (V_s) and non-saturable (V_n) components (Fig. 1A). The value for the latter was derived from the linear portion of the substrate-velocity curve between 5 and 10 mM, and is given in per millimolar units. The uptake remaining after subtraction of V_n was considered the saturable system; this was transformed to a Lineweaver-Burk plot and values of V_{max} and K_m were determined (Fig. 1B). In the case of spermidine, a much

[3] Abbreviations used: Mops, 3-[*N*-morpholino] propanesulfonic acid; MGBG, methylglyoxal (bis)guanylhydrazone; CHO, Chinese hamster ovary.

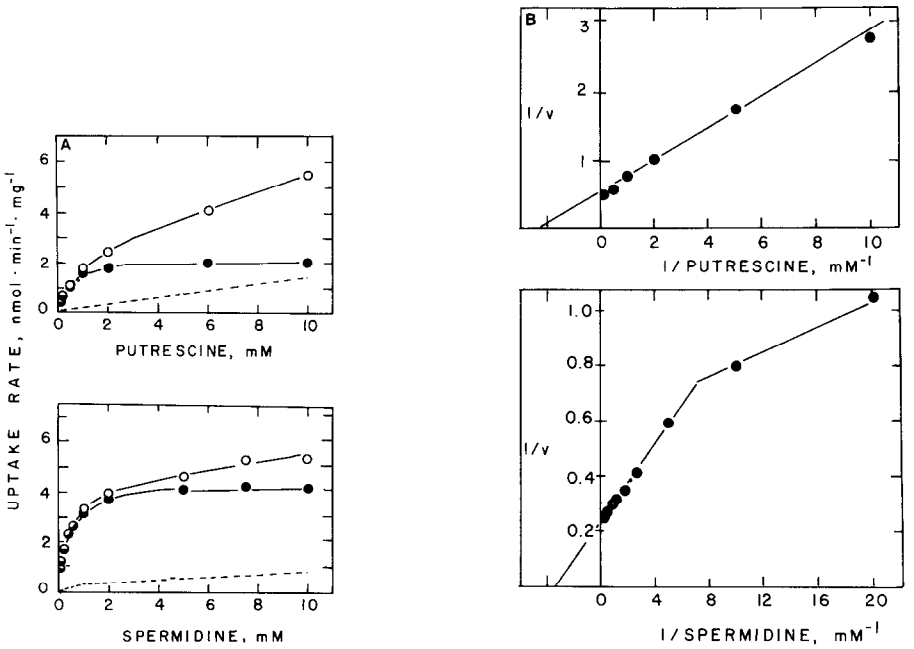

FIG. 1. (A) Concentration dependence of polyamine uptake, expressed in terms of milligrams dry weight of mycelium. Open circles, total uptake; closed circles, saturable system (after subtraction of nonsaturable component). Dashed line, uptake of comparable mycelia in Vogel's medium (separate experiment). (B) Lineweaver-Burk plots of the data for saturable systems in A.

smaller nonsaturable (or very high K_m) component was detected, and the remainder often revealed more than one saturable component. The latter could not be compared rigorously from one experiment to the next, nor between conditions, owing to the variation in the ratio of components. However, the affinity of the dominant, and often the sole, component was higher than that of the saturable component of putrescine uptake.

Uptake was nominally concentrative. With 2.54 ml intracellular water per gram dry weight (13), uptake of 40 nmol polyamine per milligram dry weight represents intracellular concentrations of ca. 16 mM. This is achieved, for instance, after 10 min in the presence of 1 mM putrescine.

From eight determinations, the values of the three uptake parameters for putrescine and spermidine, with their standard deviations, are given in Table I. The results of a single experiment with spermine are also given.

Energy Requirement

Cyanide and azide (1 mM) reduced the saturable and nonsaturable components of

TABLE I

VALUES OF UPTAKE PARAMETERS FOR PUTRESCINE, SPERMIDINE, AND SPERMINE BY *N. crassa*[a]

Polyamine	V_n[b]	V_{max}[c]	K_m (app)[d]
Putrescine	0.46 ± 0.13	3.5 ± 1.6	0.61 ± 0.12
Spermidine	0.07 ± 0.07	3.5 ± 0.86	0.24 ± 0.08
Spermine	0.09	3.2	0.07

[a] Average values ($n = 8$) for putrescine and spermidine, including mycelia of different ages. Raw (total) uptake values of mycelia exposed to 1 mM polyamine in Vogel's medium were 0.15 (putrescine) and 0.035 (spermidine) nmol min^{-1} mg (dry weight)$^{-1}$. In both cases, the uptake was largely by a non-saturable system (see text).

[b] nmol min^{-1} mg (dry weight)$^{-1}$ mM^{-1}.

[c] nmol min^{-1} mg (dry weight)$^{-1}$.

[d] mM.

polyamine uptake 75–85%, without appreciably changing the affinity of the main saturable systems for substrate. Similarly, saturable and nonsaturable uptake of 1 mM putrescine and spermidine was inhibited at least 75% at 4°C. The residual activity is not surprising in view of remaining ion gradients that might be coupled to polyamine uptake (17).

Effect of Culture Age and pH

As noted above, uptake rates increased with culture age. In the case of putrescine, the increase was about twofold during growth of the culture from 0.3 to 0.9 mg dry weight of cells per milliliter. Perhaps significantly, the increase in spermidine transport activity was only 50%, indicating again that not all parameters of uptake of the two polyamines are shared. Similarly, there was little pH dependence of putrescine uptake over the pH range 5.5–9.3. Spermidine uptake, however, showed an optimum of pH 7.2, falling three- to fourfold at pH 5.5 or 9.3. For both polyamines, the lower pH slightly increases (twofold) the affinity for substrate.

Polyamine and Nitrogen Starvation

Starvation for polyamines was imposed by transferring an ornithine-decarboxylase deficient strain (IC1894-53a) from medium supplemented with 0.5 mM putrescine to unsupplemented medium. The supplement was just enough for optimal growth, and growth stopped 4 h after the transfer. Uptake activity for putrescine and spermidine increased about 50% in the 4 h after transfer. This result cannot distinguish between an age-related increase, an induction of the transport system, and a relief of transinhibition of transport by subsiding internal polyamine pools. Nitrogen starvation was imposed on the wild-type ORS-6a strain by transferring mycelia from normal medium to medium lacking a nitrogen source. Growth was somewhat inhibited over the 4 h of starvation, and the normal increase in polyamine transport rate with age noted above did not occur.

Identity and Efflux of Internal Radioactivity

A test of the conversion of substrate to other compounds after uptake by the wild-type strain was made after short-term uptake in 1 mM putrescine (5 min) or 1 mM spermidine (15 min) or long-term incubation (60 min) with 5 mM substrate. In the case of spermidine, no compounds other than spermidine were dependably detected at the level of 1% of the material recovered from the cell, in both long- and short-term incubations. Traces of compounds that could be acetylpolyamines were detected by thin-layer chromatography, but the amounts were too small to analyze further.

In the case of putrescine, 4–5% of the internalized putrescine was converted to spermidine, as expected, in both short- and long-term incubations. Again, only traces of acetylpolyamines were detected. Putrescine gave rise to several other derivatives at low, consistently detectable levels. About 2% of the internalized putrescine in long-term incubations was acid insoluble, presumably covalently linked to macromolecules. Of the soluble material in the same incubation, 3–5% of the radioactivity was less basic than acetylputrescine or putrescine. These materials were not studied further. In sum, only 10% or less of the radioactivity entering as putrescine was converted to other forms even in heavily labeled cells.

Mycelia loaded with ^{14}C-polyamines were tested for efflux of radioactivity. Cells were preloaded for 2 min with 0.3 mM putrescine or 0.1 mM spermidine, or for 20 min with 5 mM putrescine or 5 mM spermidine. After removing the external radioactivity by washing them in 0.25 M NaCl, cells were transferred to fresh Mops-glucose incubation medium. Samples were transferred in parallel to Mops-glucose medium containing 5 mM cognate, unlabeled polyamine. Efflux of [^{14}C]putrescine was 1 or 8% of the uptake rate at low and high preloading, respectively, and this was not significantly increased in the presence of 5 mM exogenous putrescine. Spermidine efflux was 3% of the uptake rate in heavily

TABLE II

EFFLUX OF INTERNALIZED ^{14}C-POLYAMINES[a]

^{14}C-Substrate loaded	Efflux conditions			^{14}C-polyamine distribution	
	Polyamine (5 mM)	Butanol	Time (min)	Medium	Cell pellet
Putrescine	–	–	0	8	92
	–	–	5	11	89
	+	–	5	15	85
	–	+	5	77	23
	+	+	5	95	5
Spermidine	–	–	0	6	94
	–	–	5	8	92
	+	–	5	19	81
	–	+	5	35	65
	+	+	5	99	1

[a] Cells were loaded using 2 mM ^{14}C-polyamines under standard conditions. They were washed and transferred to the standard uptake medium, where efflux was tested with additions of the cognate polyamine or with *n*-butanol (7.5%, single phase). A parallel, double-label experiment showed that *n*-butanol removes all acid-soluble [^{3}H]arginine from cells.

preloaded cells, and this was stimulated twofold by external 5 mM spermidine. The dilution of internalized radioactivity by endogenous pools was insignificant in this case. In cells with low preloading by [^{14}C]-spermidine, the dilution may have been as much as fivefold, and this may have contributed to the lack of detectable efflux. This was undoubtedly minimized, however, by the known compartmentation of endogenous spermidine (7).

Efflux of polyamines from permeabilized cells was tested. Cells were treated during growth with tracer levels of [^{3}H]arginine to label the vacuolar pool of this amino acid (18), and, after transfer to Mops-glucose, they were loaded with 2 mM ^{14}C-polyamine. After washes with 0.25 M NaCl in Mops-glucose, the cells were incubated for 5 min in Mops-glucose containing either 5 mM unlabeled polyamine, 7.5% *n*-butanol, or both (Table II). Little efflux of polyamines from intact cells was seen without added polyamine; addition of polyamine led to slightly greater efflux. Butanol led to the removal of 77% of the [^{14}C]putrescine and 35% of the [^{14}C]spermidine from the cell pellet. When both butanol and cold polyamines were present, over 95% [^{14}C]-putrescine and 99% of the [^{14}C]spermidine was driven from the pellet. (The remaining putrescine may have been acid insoluble; see above.) All acid-soluble arginine was removed from the cell pellet by butanol treatment, whether or not unlabeled polyamine was present, showing that cell permeabilization was complete. The results show that the cell membrane is required to retain polyamines in a nonexchangeable or slowly exchangeable fashion, and that polyamines are bound exchangeably to cell constituents after permeabilization. The last point does not of itself prove, however, that polyamines are similarly bound in intact cells.

Inhibitors

Competition among polyamines, and between polyamines and certain other compounds, was tested by presenting the latter simultaneously with ^{14}C-polyamines. A subsaturating concentration (0.2 mM) of substrate ^{14}C-polyamines was used, at

TABLE III

INHIBITION OF POLYAMINE UPTAKE BY POLYAMINES

Inhibitor	Concn (mM)	Percentage normal uptake[a] at 0.2 mM substrate [^{14}C]Put	[^{14}C]Spd	[^{14}C]Spm
Putrescine	0.2	78 (161)[b]	81	82
	2.0	41 (407)	52	53
Spermidine	0.2	31	77 (153)	86
	2.0	19	21 (231)	39
Spermine	0.2	28	25	51 (100)
	2.0	16	8	13 (145)

[a] Normal uptake (100%) was determined in parallel in standard Mops-glucose buffer with no additions.

[b] The figures for "inhibition" by cognate polyamines were determined without recalculating specific radioactivities, to make them comparable to figures for other polyamines. Figures in parentheses indicate the actual percentage of control values in nanomoles; as expected, additional cognate polyamine stimulated uptake mainly by increasing uptake via the nonsaturable transport component.

which 65% of putrescine uptake and 96% of spermidine uptake was by saturable system(s). Equimolar concentrations of the polyamines were mutually inhibitory (Table III), suggesting that they shared components of uptake. The inhibitory series (spermine > spermidine > putrescine) was in accord with their decreasing affinities as substrates of their saturable systems (Table I). Increasing the competitor concentration to 2 mM led to further inhibition; this was minimized to some extent by operation of the nonsaturable uptake system, and hence the large residual uptake of labeled polyamine. A titration of the uptake of 0.2 mM [^{14}C]putrescine and [^{14}C]-spermidine by unlabeled spermine is shown in Fig. 2. The similarity of the two curves suggests again that components of uptake for all three polyamines are shared. Spermidine behaved as a competitive inhibitor of putrescine uptake (Fig. 3A), with K_i values in the range of the K_m's for spermidine uptake (0.06–0.2 mM). The polyamine pathway inhibitor methylglyoxal (bis)guanylhydrazone (MGBG) was weakly inhibitory ($I_{0.5}$ = ca. 1 mM) to the uptake of both putrescine and spermidine (Fig. 2).

Inhibition of uptake of 0.2 mM ^{14}C-polyamine by equimolar L-arginine and L-ornithine was 20% for putrescine and 50% for spermidine; inhibition did not significantly increase at 2.0 mM competitor. The uptake of arginine and ornithine by the basic amino acid transport system (K_m = 2 $\times 10^{-6}$ to 1 $\times 10^{-5}$ M) is normally saturated at the lower amino acid concentration. The inhibition by the amino acids may reflect competition for the driving force of the membrane potential, anti- or symport ion, or metabolic energy.

Uptake from the Growth Medium

Vogel's medium N has 86 mM monvalent cation, 0.8 mM Mg^{2+}, and 0.7 mM Ca^{2+}. Uptake of ^{14}C-polyamines from Vogel's medium is therefore greatly reduced, compared to the same cells in the standard incubation medium (Fig. 1A and Table I, legend). The saturable components of uptake of both polyamines are almost wholly

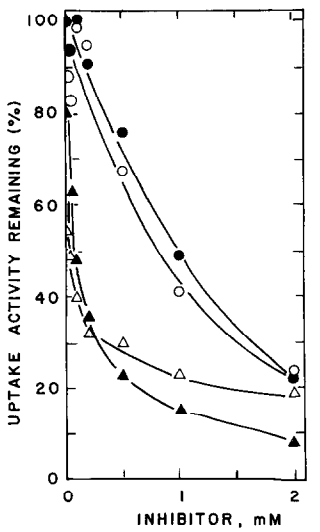

FIG. 2. Effects of methylglyoxal (bis)guanylhydrazone (circles) and spermine (triangles) on the uptake of 0.2 mM putrescine (open symbols) and 0.2 mM spermidine (closed symbols).

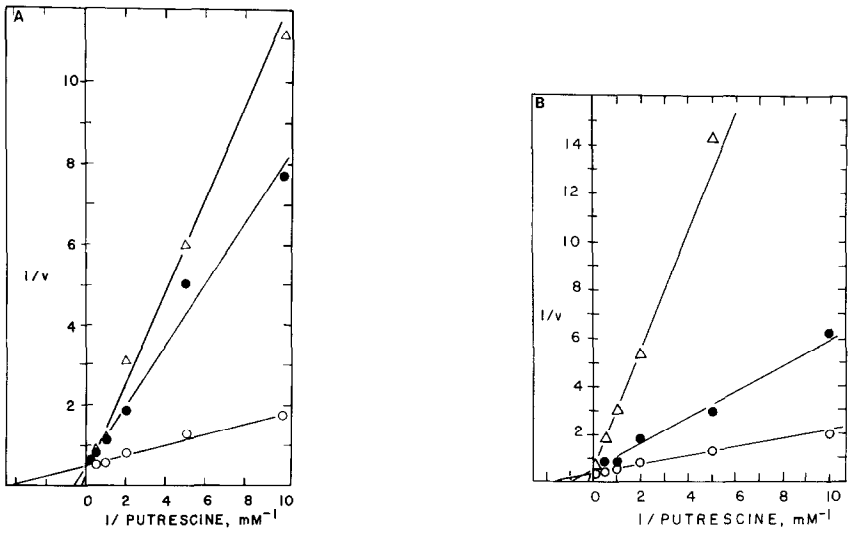

FIG. 3. Lineweaver-Burk plots of data showing the effects of spermidine (A) and Ca^{2+} (B) on putrescine transport. In A, the spermidine concentrations used are nil (open circles), 0.2 mM (closed circles), and 2.0 (triangles). In B the CaCl concentrations used are nil (open circles), 0.025 mM (closed circles), and 0.15 mM (triangles).

lost, and even the nonsaturable component of putrescine is severely inhibited. Inhibition is not exerted by 7.5% sorbitol (ca. 0.5 OsM, over twice that of Vogel's medium), showing that the inhibition by medium components is due to cation concentration or ionic strength, not osmotic potential. In fact it is known that the membrane potential of cells in cation-rich media such as Vogel's medium is lower than that in the dilute medium we have used for uptake studies, and this too may account for the low rates of uptake in the growth medium (19).

Preincubation with Cations

The effect of individual growth medium components upon polyamine uptake was determined. Additions of cation were usually made 30 min prior to the addition of labeled substrate. This regularized the outcome, but most immediate effects were similar. Calcium was a strong inhibitor of uptake of 0.2 mM putrescine and spermidine, where saturable systems predominate. The nonsaturable components were not affected; for the saturable putrescine uptake system, inhibition was nominally competitive (K_i = ca. 0.01–0.02 mM) (Fig. 3B). Ca^{2+} was somewhat less effective on the saturable components of spermidine uptake (data not shown). Mg^{2+} (0.15–0.5 mM) had no effect, or slightly stimulated uptake of both polyamines.

Monovalent cations (Na^+, NH_4^+, and K^+) inhibited putrescine and spermidine uptake when added with substrate. The half-inhibitory concentration was about 40–50 mM additional cation, compared to the standard conditions of 20 mM Na^+. Inhibition was complete at 200 mM. Putrescine uptake in the presence of 60 mM K^+ was largely mediated by the nonsaturable system (Fig. 4). Under the same conditions, spermidine uptake was inhibited, and the apparent affinity of the remaining saturable component for substrate was lower (Fig. 4).

Manipulation of K^+

In order to gain further understanding of the relation of putrescine uptake to cellular cations, we manipulated the intramycelial concentration of the predominant

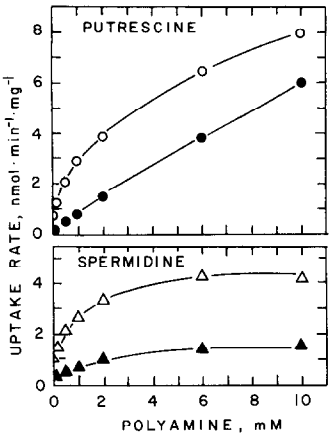

FIG. 4. Effect of 60 mM KCl on the uptake of putrescine (top) and spermidine (bottom), expressed in terms of milligrams dry weight of mycelium. The polyamine concentrations vary from 0.1 to 10 mM (abscissa). Open symbols, control; closed symbols, +60 mM KCl.

cellular cation, K^+ (Table IV). This was done by growing cells in 0.1 mM K^+, leading to a deficient K^+ pool and inducing a high-affinity K^+ transport system (20). Starved cells were compared to cells grown in the normal medium or to K^+-starved cells to which 1 mM KCl had been restored 10 min before harvesting and testing. We also tested the effect of the preincubation period (10 vs 40 min) prior to the uptake period, and the cation constitution (20 mM Na^+, 20 mM K^+, or no cation) of the preincubation medium. The mycelia were tested for transport activity with 1 mM putrescine. The data (Table IV) show, first, that cells harvested from the normal medium are reasonably stable with time in the standard (pre)incubation medium (with Na^+), and incubation in K^+-containing medium leads to similar values. In distilled water, however, the cells have more than twofold the normal transport activity, which increases during preincubation. The increase in the absence of Na^+ is consistent with the inhibition of transport by this ion.

K^+-starved cells showed greatly increased transport capacity, which decreased with time of preincubation in K^+ Mops-glucose to normal or less than normal levels. Restoration of K^+ to the growth medium normalizes the cells if transport is tested at 10 min preincubation, but further loss of transport activity still occurs after another 30 min preincubation. Polyamine determinations show that K^+ starvation leads to less than a doubling of the putrescine and spermidine pools (normally 0.8 and 17 nmol/mg, dry weight), and that there is no simple correlation between internal polyamine pools and the rate of putrescine transport.

DISCUSSION

Our data indicate that polyamine transport in the standard, dilute buffer is concentrative and requires energy and an intact cell membrane. Part of the concentrative character of uptake may reflect binding to cell constituents, as the experiment with permeabilized cells shows. There is a prominent diffusional component of putrescine uptake, also energy dependent, that is greater for putrescine than for spermidine. This system is about one-half as active in Vogel's medium as in the standard conditions of uptake, and is

TABLE IV

PUTRESCINE TRANSPORT RATES OF MYCELIA WITH ALTERED K^+ STATUS

Uptake conditions[a]		Uptake rate (nmol min^{-1} mg^{-1}) of mycelia[b]		
Preincubation time (min)	Medium	Normal	K^+ starved	K^+ restored
10	Na^+-Mops	1.9	3.7	2.2
	K^+-Mops	1.1	4.1	1.9
	Water	3.9	8.7	4.9
40	Na^+-Mops	2.0	4.1	1.9
	K^+-Mops	1.8	1.0	1.0
	Water	5.1	2.7	1.3

[a] Preincubation was done in the uptake media noted.

[b] Cells were grown in Vogel's medium, normal or low K^+ (0.1 mM), as noted under Materials and Methods. The K^+-restored cells were grown in low-K^+ medium, but were given 1.0 mM KCl 10 min before harvest.

the sole putrescine transport mechanism of mycelia during growth in Vogel's medium. [The diffusional component(s) may actually be saturable, but have such a low affinity for polyamines that they show linear concentration dependence up to 20 mM substrate]. Steady-state studies in progress indicate that external putrescine in the growth medium merely equilibrates with cell water, and that the concentration gradient seen for spermidine in growing cells may be due almost wholly to intracellular binding. These observations are consistent with a largely diffusional uptake of both polyamines from the growth medium. The low level of polyamine transport in Vogel's medium accounts for the high polyamine concentrations needed to grow polyamine-dependent mutants such as strain IC1894-53a (21).

Some components of the uptake process for the three polyamines are shared. Each polyamine inhibits the uptake of others, in rough correspondence with their affinities as substrates. Their similar inhibition by Ca^{2+}, MGBG, and monovalent cation, the first being competitive or partially competitive, reinforces this view. However, their differing response to pH and the different rates of increase of uptake activity with the age of the culture show that the uptake processes for the two polyamines have distinct properties. The complex concentration dependence of spermidine suggests that several different saturable components may prevail, only the major one of which may also transport putrescine. The data are not adequate to answer this question; mutants will allow us to distinguish common and distinctive elements.

Another question pertains to the driving force of uptake. Azide and cyanide, which deprive the cell of ATP within a minute (17), and thus the means to maintain a high membrane potential, inhibit the uptake of polyamines, but not completely. The saturable and nonsaturable components of putrescine uptake are both inhibited about 70%, and the saturable system for spermidine is even more affected. The residual uptake may reflect the use of the residual membrane potential derived from ion gradients, especially K^+, that remain after ATP is gone. These gradients are maintained in part by lowered permeability of the membrane to leakage of intracellular ions under these conditions (22).

Starvation for a variety of nutrients leads to reduction in ion leaks, and therefore to a high membrane potential if ATP is available. This may explain first the high uptake under our standard conditions (Mops–glucose) and the effect of polyamine and K^+ starvation. Moreover, the membrane potential is known to be low in Vogel's growth medium, and external K^+ lowers the membrane potential (19). Our data also suggest that high external monovalent cations interfere with polyamine binding. Finally, the possibility that K^+ is an antiport substrate, or that K^+ ejection must accompany polyamine uptake (23) is not ruled out. Certainly the uptake of polyamines must be accompanied by entry of anions or exit of cations. Because K^+ is the predominant cation in *N. crassa* cells, its discharge upon polyamine uptake would be expected.

Vogel's medium inhibits polyamine uptake for several reasons, three of which are documented here: the high monovalent cation concentration; the presence of Ca^{2+}; and the lower pH (5.5) than the standard uptake medium (7.2), a consideration applying only to spermidine. This does not mean the uptake system is without biological significance; in nature, *N. crassa* encounters many environments that are likely to permit concentrative uptake. However, neither polyamine nor nitrogen starvation greatly stimulates polyamine transport, and it is therefore debatable whether the systems in *N. crassa* have any significance for polyamine or nitrogen utilization. Because *N. crassa* makes its own polyamines, because its affinities of transport of polyamines are relatively low, and because it does not use polyamines as nitrogen sources (R. H. Davis and J. L. Ristow, unpublished observations), a specific polyamine transport system would not appear to be essential. The systems may have

evolved for the uptake of other materials entirely.

The best characterized polyamine transport systems among microorganisms are those of the bacterium *Escherichia coli* (5, 24, 25), in which distinct putrescine and spermidine/spermine systems were discerned. Among true fungi, polyamine transport has been studied in *Aspergillus nidulans* (26). Transport was measured in the growth medium, and the K_m's are rather high (1–4 mM) for putrescine and spermidine. This may reflect competitive inhibition by inorganic cations in the medium, as we have shown here for *N. crassa*. In *A. nidulans*, spermidine and spermine do not compete with putrescine for uptake, but putrescine appears to compete with spermidine uptake. No diffusional component was remarked upon.

Putrescine uptake by the cellular slime mold, *Dictyostelium discoideum* reveals multiple components (27). A saturable system with high affinity is inhibited by spermidine and spermine. Another system is nonsaturable and less inhibited by the other polyamines, much as we observe in *N. crassa*. Efflux was minimal, even in the presence of unlabeled putrescine. A unified explanation offered for both systems was that putrescine was taken into the cell by pinocytosis, after cell-surface binding (the saturable component) and membrane internalization. At higher concentrations, pinocytosis would effect significant, nonsaturable uptake in the fluid phase. Similar as the putrescine uptake data of Turner *et al.* (27) are to ours, we do not believe that pinocytosis is significant in *N. crassa*. This is supported by the fact that the nonsaturable component of spermidine uptake is at least fivefold lower than that for putrescine.

Higher eucaryotic cells vary considerably with respect to kinetics of polyamine uptake. In rabbit lung slices, putrescine uptake resembles that of *N. crassa* in having a considerable nonsaturable component, inhibition by spermine, and inhibition by Na^+ (28). Another study on perfused rat lung (29) indicates that spermidine is transported by a saturable system which is inhibited strongly by spermine and putrescine, and more weakly by MGBG. A more complex picture has been presented by Byers *et al.* (30), working with CHO and rat lung cells. In the former cell type, mutation to resistance to MGBG eliminated the ability to transport any of the polyamines. This indicated a common component for all, and indeed, putrescine and spermine both inhibited spermidine uptake. Results on the uptake of polyamines by isolated petals of *Saintpaulia ionantha* (31) indicate that uptake is inefficient in terms of affinity (K_m's > 1 mM), and that nonsaturable components are found for both putrescine and spermidine. Putrescine and spermidine uptake were not inhibited by equimolar levels of the other, nor was either polyamine sensitive to inhibition by Ca^{2+} or Mg^{2+}.

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