Putrescine Uptake by the Cellular Slime Mould *Dictyostelium discoideum*

By REBECCA TURNER, MICHAEL J. NORTH* and JENNIFER M. HARWOOD
Department of Biochemistry, University of Stirling, Stirling FK9 4LA, Scotland, U.K.

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1. Rapid labelling occurs when myxamoebae of *Dictyostelium discoideum* strain AX2 are incubated with [1,4-¹⁴C]putrescine. Labelling is energy-dependent. 2. The label enters a pool from which rapid exchange with extracellular putrescine does not occur, and labelling is believed to represent uptake into the cells. 3. The concentration-dependence of putrescine uptake indicates that a number of systems are involved, at least one of which is saturable, with a K_m of 9.1 μ M-putrescine. At high putrescine concentrations the overall uptake process is non-saturable. 4. Significant metabolism of putrescine and loss of intracellular putrescine to the medium only occurred when cells were incubated with millimolar concentrations of extracellular putrescine. 5. Putrescine uptake was inhibited by diamines, polyamines, bivalent metal ions and ω -aminocarboxylic acids. 6. The ability to take up putrescine at low concentrations decreased during starvation of myxamoebae. 7. The results are interpreted in terms of a model for putrescine uptake involving adsorptive pinocytosis at low concentrations and fluid-phase pinocytosis at high concentrations.

The life cycle of the cellular slime mould *Dictyostelium discoideum* is divided into two mutually exclusive phases, a vegetative phase, in which the single-celled myxamoebae feed and multiply, and a developmental phase, during which the myxamoebae, now starved of food, aggregate together and undergo a series of morphogenetic and biochemical changes which culminate in the formation of fruiting bodies (Loomis, 1975). The relative simplicity of this developmental phase makes the organism an attractive model system for studying differentiation. During both phases of the life cycle the cells are permeable to various materials including small molecules such as nutrients, labelled precursors and inhibitors. The processes involved may not only be important for the uptake of extracellular materials, but similar processes could also be involved in the loss of metabolites from the cells during starvation.

Although Ryter & de Chastellier (1977) have stated that *D. discoideum* exclusively feeds by pinocytosis and phagocytosis, very little has actually been published on the mechanism of uptake of small molecules. From studies of the effects of 2,4-dinitrophenol and low temperature, Lee (1972c) concluded that inulin uptake occurs by pinocytosis, whereas lysine and glutamate enter the cells by passive diffusion. Studies by North & Williams (1978) have demonstrated that the uptake of 6-aminohexanoic acid (ϵ -aminocaproic acid), an inhibitor of development, is sensitive to 2,4-dinitrophenol and NaN₃, and

Abbreviation used: Mes, 4-morpholine-ethanesulphonic acid.

* To whom requests for reprints should be sent.

is also inhibited by cycloheximide. It was suggested that its uptake involved pinocytosis. Similar inhibition had been observed for glucose uptake and, in contrast with the findings of Lee (1972c), for lysine uptake. Uptake of 6-aminohexanoic acid occurs by a non-saturable process and is non-concentrative. Lee (1972c) also found that the amount of glutamate and lysine taken up in his experiments was comparable with that expected for equilibrium of the internal pool with the suspending medium.

During an investigation of polyamine metabolism in *D. discoideum* we investigated the uptake of another small molecule, putrescine (1,4-diaminobutane), but the characteristics of this process differed markedly from those observed for the uptake of 6-aminohexanoic acid and the other amino acids. Putrescine is a major cell constituent and is present in myxamoebae at a concentration equivalent to 14 mM (North & Turner, 1977). It can be synthesized by the cells from ornithine (Turner & North, 1977), but may also be taken up from the surrounding medium. In this paper we present the results of a study of putrescine uptake and show that extracellular putrescine can be concentrated by the cells at a rapid rate. The properties of the uptake process suggest that it occurs by adsorptive pinocytosis.

Materials and Methods

Organism and growth conditions

Unless otherwise indicated *D. discoideum* strain AX2 (A.T.C.C. 24397) was used. Myxamoebae were grown as described by Watts & Ashworth (1970) in

HL5 medium containing no added sugar (NS cells). In some experiments 86mM-glucose was added to the growth medium (G cells).

Measurement of putrescine uptake

Myxamoebae were harvested from the growth medium during the exponential phase of growth (at a density of 2×10^6 – 4×10^6 cells/ml for NS cells, 4×10^6 – 8×10^6 cells/ml for G cells) by centrifugation at 500g for 10 min, washed once with water and finally resuspended in 17mM-sodium phosphate buffer (either pH6.0 or pH7.0) at a density of between 1×10^6 and 5×10^6 cells/ml. Unless otherwise indicated 10ml suspensions were used. The suspension was incubated at 22°C in an orbital incubator (130 rev./min) and after a variable preincubation period (normally 15 min) a sample of [1,4- 14 C]putrescine was added. To measure the labelling of cells samples were withdrawn at regular intervals, centrifuged in a Jobling model 320 microcentrifuge for 1 min, and then the cells were washed twice with water and finally resuspended in 2ml of water. The suspensions were transferred to vials together with 10ml of scintillation fluid and the radioactivity was determined in a Philips liquid-scintillation counter exactly as described previously (North & Williams, 1978). Rates of putrescine uptake were determined as nmol of putrescine/min per 10^7 cells.

Analysis of the distribution of radioactivity in labelled cells

Myxamoebae, labelled with [14 C]putrescine as described above, were harvested by centrifugation (500g for 1 min), washed and resuspended in water. HClO₄ was added to give a final concentration of 2% (w/v) and the sample was stood at 0°C for 30 min. It was then centrifuged (1000g for 5 min) and the pellet washed once with 2% (w/v) HClO₄. The supernatants were combined and retained for further analysis.

Radioactivity in the pellet was measured by resuspending it in 2% (w/v) HClO₄, collecting the acid-insoluble material on a Whatman GF/C filter and washing with 10ml of HClO₄ and then 10ml of ethanol. The dried filter was counted for radioactivity as described above.

The acid-soluble material was fractionated on a Dowex 50 (200–400 mesh; 8% cross-linked) column (100mm × 10mm) (Turner & North, 1977). The combined supernatants were applied to the column and the unbound material was collected (fraction 1). The column was then washed with 25ml of 0.05M-HCl and the eluted material collected (fraction 2). The final fraction (fraction 3) was obtained by eluting with 75ml of 6M-HCl. This fraction contained putrescine and the other major cellular polyamines, 1,3-

diaminopropane and spermidine. Radioactivity was measured in samples of each fraction as described above.

Some fractions were analysed further by electrophoresis. Each sample was concentrated by rotary evaporation at 60°C, and the residue redissolved in 100–200 μ l of 0.02M-HCl. Portions (20 μ l) were applied to strips of Whatman chromatography paper (0.33mm thick, 30mm wide), and electrophoresis was carried out in 65mM-5-sulphosalicylic acid, adjusted to pH3.2 with NaOH solution, for 2h at 240V (North & Turner, 1977). The strips were dried at room temperature and then scanned for radioactivity by using a Berthold Dünnschicht scanner II. The positions of standard polyamines were determined by staining with ninhydrin reagent (Inoue & Mizutani, 1973).

Materials

Putrescine dihydrochloride, cadaverine dihydrochloride, spermidine trihydrochloride, spermine tetrahydrochloride, β -alanine, 4-aminobutanoic acid, 6-aminohexanoic acid, 8-amino-octanoic acid and Dowex 50 were supplied by Sigma (London) Chemical Co., Poole, Dorset, U.K. 2,4-Dinitrophenol, NaN₃, 1,3-diaminopropane and 5-sulphosalicylic acid were products of BDH Chemicals, Poole, Dorset, U.K., and 5-aminopentanoic acid was a product of Aldrich Chemical Co. Ltd., Gillingham, Dorset, U.K.

[1,4- 14 C]Putrescine dihydrochloride was supplied by The Radiochemical Centre, Amersham, Bucks., U.K., and 6-amino[1- 14 C]hexanoic acid by New England Nuclear, Winchester, Hants., U.K.

All other chemicals used were of the highest purity available from either BDH Chemicals or Fisons, Loughborough, Leics., U.K.

Results

Kinetics of [14 C]putrescine labelling

Fig. 1 shows the time course of labelling of myxamoebae (NS cells) during incubation with [14 C]putrescine in 17mM-sodium phosphate buffer at pH7.0. With 1 μ M-putrescine, labelling was rapid, and within 1h over 20% of the putrescine present in the buffer became associated with the cells. The volume occupied by the cells was approx. 0.15% of the total volume of the suspension. For incubation times of 30 min or less the labelling was proportional to the cell density up to a density of 5×10^6 cells/ml. At higher putrescine concentrations the proportion of extracellular putrescine associated with the cells after a given time was smaller. The pH had little effect on the rate of labelling in the range from 6.0 to 7.5, and rates were measured routinely at either

pH 6.0 or pH 7.0. Similar rates to those measured with NS cells were obtained with G cells.

At both high and low concentrations of putrescine, labelling was considerably reduced at low temperature and was sensitive to NaN_3 and to 2,4-dinitrophenol (Table 1). The latter was effective at pH 6.0 but not

at pH 7.0. A reversal of dinitrophenol inhibition by increased intracellular pH has also been reported by Geller & Brenner (1978) in studies on oscillations in *D. discoideum*. The results in Table 1 indicate that labelling involved an active process and could not have been due solely to the binding of [^{14}C]putrescine to the cell surface. This was also indicated by the following observations. When myxamoebae were preincubated with excess (up to 1000-fold) unlabelled putrescine to saturate any surface binding sites, the rate of labelling, measured after the cells had been washed and incubated with labelled putrescine, was no less than in untreated controls. If labelling were due solely to binding at the cell surface, unlabelled putrescine bound during preincubation must have either been removed by the washing (with water) or exchanged very rapidly with the labelled putrescine. However, when labelled cells were washed with water, 1 mM-putrescine, 200 mM- NaCl , 5 mM- MgCl_2 or 5 mM- CaCl_2 , the radioactivity remained associated with the cells, and if labelled cells were incubated for long periods in buffer containing unlabelled putrescine, only a small portion of the radioactivity was recovered in the medium (see Fig. 3). The labelled putrescine had entered a pool from which rapid exchange with extracellular putrescine was not possible. This pool must have been intracellular, and labelling must have represented uptake into the cells.

A more detailed study of the dependence of the uptake rate on putrescine concentration revealed that at least one, and probably more, saturable systems were involved. A double-reciprocal plot (Lineweaver & Burk, 1934) of uptake data for a low putrescine-concentration range (Fig. 2a) gave a straight line, defining a system with an apparent K_m for putrescine of $9.1\text{ }\mu\text{M}$ and a maximum velocity of $0.123\text{ nmol/min per }10^7\text{ cells}$. With higher putrescine concentrations rates were even faster than this, showing that other systems were involved. However, it was not possible

Fig. 1. Time course of labelling of myxamoebae with [^{14}C]putrescine
Myxamoebae were incubated at pH 7.0 at a density of 3.3×10^6 cells/ml. After 2 h [^{14}C]putrescine was added at concentration of (a) $1\text{ }\mu\text{M}$ ($5.90\text{ }\mu\text{Ci}/\mu\text{mol}$) or (b) 2 mM ($3.10\text{ }\mu\text{Ci}/\text{mmol}$). Labelling was measured as described in the Materials and Methods section.

Table 1. Effects of 2,4-dinitrophenol, NaN_3 and low temperature on [^{14}C]putrescine labelling
Myxamoebae were incubated at densities of between 2.5×10^6 and 4.0×10^6 cells/ml. After 15 min [^{14}C]putrescine was added and labelling determined as described in the Materials and Methods section. Additions were made at the same time as putrescine. The labelling rate is given as a percentage of that for myxamoebae incubated at the same cell density at pH 7.0 and 22°C .

Addition to buffer	Temperature ($^\circ\text{C}$)	pH	Putrescine labelling (%)	
			1 μM -Putrescine	5 mM-Putrescine
None	22	6.0	95	131
None	22	7.0	100	100
0.1 mM-Dinitrophenol	22	6.0	0	5
0.1 mM-Dinitrophenol	22	7.0	100	100
1.0 mM- NaN_3	22	7.0	8	0
None	0	7.0	10	17

to fit the data from higher concentration ranges to straight-line double-reciprocal plots, and at millimolar concentrations these plots curved towards the origin (Fig. 2*b*). This suggests that a non-saturable process made a significant contribution to the overall uptake at high concentrations.

Fate of labelled putrescine

When myxamoebae were incubated with 0.1 mM- $[^{14}\text{C}]$ putrescine for 4 h, subsequent analysis showed that over 99% of the cellular radioactivity was present in fraction 3 eluted from the Dowex 50 column with 6M-HCl (Table 2), the fraction containing putrescine. With 10 mM- $[^{14}\text{C}]$ putrescine

there was a significantly greater proportion of radioactivity in other fractions, including the acid-insoluble fraction, and so putrescine metabolism must have taken place. Nevertheless over 90% of the radioactivity was still recovered in the putrescine-containing fraction. This fraction, which also contained spermidine, a product of putrescine metabolism (Turner & North, 1977), was further analysed by paper electrophoresis. Some radioactivity migrated with spermidine, but most was detected in the faster position characteristic of putrescine.

If labelled cells were washed and transferred to fresh buffer containing unlabelled putrescine, more

Fig. 2. *Lineweaver-Burk plots for putrescine uptake*

Myxamoebae were incubated at pH 7.0 at a density of 2.5×10^6 cells/ml. After 15 min $[^{14}\text{C}]$ putrescine (0.05 $\mu\text{Ci/ml}$) was added at concentrations between (a) 1 and 20 μM or (b) 25 μM and 20 mM, and the uptake rate determined.

Table 2. *Distribution of radioactivity in cell fractions after incubation with $[^{14}\text{C}]$ putrescine*

Myxamoebae were incubated at pH 7 at a density of 4.4×10^6 cells/ml with $[^{14}\text{C}]$ putrescine (0.1 $\mu\text{Ci/ml}$) at the concentration indicated. After 4 h the cells were harvested, washed twice with water and fractions (1, 2 and 3) prepared and analysed for radioactivity as described in the Materials and Methods section. Over 90% of the acid-soluble radioactivity applied to the Dowex column was recovered.

[Putrescine] (mM)	Radioactivity in acid-insoluble material (% of total in cells)	Radioactivity in Dowex-column fractions (% of total recovered)		
		1	2	3
0.1	0.4	0.04	0.4	99.6
1.0	1.2	0.3	0.6	99.1
10.0	3.7	0.5	5.1	94.4

Table 3. *Distribution of radioactivity in cell fractions after a 'chase' with unlabelled putrescine*

Myxamoebae were incubated at a density of 8×10^6 cells/ml in 100 ml of 17 mM-sodium phosphate buffer, pH 7.0. After 15 min [^{14}C]putrescine ($1.6 \mu\text{M}$; $62 \mu\text{Ci}/\mu\text{mol}$) was added and incubation was continued for 15 min. The cells were harvested, washed twice with water and resuspended at a density of 4.4×10^6 cells/ml in 10 ml portions of 17 mM-sodium phosphate buffer, pH 7.0, containing unlabelled putrescine at the concentration indicated. After 4 h the cells were harvested and fractions (1, 2 and 3) prepared and analysed for radioactivity as described in the Materials and Methods section. Over 90% of the acid-soluble radioactivity applied to the Dowex column was recovered.

[Putrescine] (mM)	Total radioactivity in cells (% of initial value)	Radioactivity in acid- insoluble material (% of total in cells)	Radioactivity in Dowex- column fractions (% of total recovered)		
			1	2	3
Initial	100	0.04	0	0.1	99.9
0	100	0.27	0.05	0.5	99.4
0.1	90.5	0.51	0.01	1.3	98.7
1.0	54.8	2.18	0.1	3.9	96.0
10.0	23.9	8.53	1.5	37.1	61.4

Fig. 3. *Efflux of radioactively labelled material from myxamoebae prelabelled with [^{14}C]putrescine*

The cells were labelled with [^{14}C]putrescine exactly as described in the legend of Table 3. Suspensions of cells (10 ml) at 4.4×10^6 cells/ml in 17 mM-sodium phosphate buffer, pH 7.0, containing the additions listed below, were incubated. At hourly intervals 1 ml samples were withdrawn and centrifuged for 5 min at 500g. The supernatant was transferred to a scintillation vial and counted for radioactivity. (a) No addition ($\circ$); 0.1 mM-putrescine ($\bullet$); 1.0 mM-putrescine ($\square$); 10 mM-putrescine ($\blacksquare$). (b) 1.0 mM-1,3-diaminopropane ($\circ$); 1.0 mM-cadaverine ($\bullet$); 1.0 mM-spermidine ($\square$); 1.0 mM-spermine ($\blacksquare$).

radioactivity could be chased into non-putrescine-containing fractions (Table 3). However, after 4 h of incubation with 10 mM-putrescine over 50% of the radioactivity remained in the putrescine-containing fraction. Electrophoretic analysis showed that most of this radioactivity migrated with putrescine and only a small amount corresponded to spermidine. Analysis of fraction 2 (eluted from Dowex 50 with

0.5M-HCl), which contained 37% of the acid-soluble radioactivity (34% of the total), revealed two other as yet unidentified compounds, one of which was positively charged but moved more slowly than putrescine, spermidine or spermine, while the other remained at the origin. No labelled putrescine was present in this fraction. With lower concentrations of extracellular putrescine much less

radioactivity appeared in the non-putrescine fractions, and in its absence over 99% of the cellular radioactivity was recovered as putrescine.

As the concentration of extracellular putrescine used for the chase was increased, so less total radioactivity was recovered with the cells (Table 3), indicating a loss of putrescine or its metabolites from the cells. Some of the lost radioactivity was recovered in the buffer. Fig. 3(a) shows a concentration-dependent efflux of radioactive material from the cells. With putrescine concentrations below 0.1 M the net efflux was insignificant. The influx of radioactivity observed when no putrescine was added was due to the uptake of [^{14}C]putrescine not removed during washing. In other cultures this influx would have been negligible because of the dilution by unlabelled putrescine. At 10 mM-putrescine an efflux rate corresponding to a loss of 2.9% of the initial cellular radioactivity per h was measured. Electrophoretic analysis of the buffer indicated that most of the radioactive material was putrescine, although a small proportion ran in a position characteristic of slower-moving material detected in the corresponding cell extracts. With lower extracellular putrescine concentrations, all the radioactivity in the buffer corresponded to putrescine.

Despite the presence of labelled material in the buffer, the amount was insufficient to account for all of the radioactivity lost from the cells. A similar net loss has been observed from labelled cells undergoing development on Millipore filters (Turner & North, 1977). The loss can be accounted for by the production

of $^{14}\text{CO}_2$ which is evolved by myxamoebae labelled with [^{14}C]putrescine (R. Turner, C. M. McDonald & M. J. North, unpublished work).

Efflux of radioactive material from labelled cells was stimulated by other diamines and polyamines (Fig. 3b). In all cases the major labelled material in the buffer after 4 h corresponded to putrescine, although with added spermidine some of the excreted radioactivity was in the form of spermidine. No efflux of radioactivity material occurred with 10 mM- MgCl_2 , - CaCl_2 or - MnCl_2 .

Inhibitors of putrescine uptake

Putrescine uptake was reduced when one of a number of compounds was added to the suspension buffer. Polyamines and diamines were all inhibitory, with complex inhibition kinetics. Spermine and spermidine had a severe effect on uptake at low putrescine concentrations, eliminating the saturable components, but they had less effect at higher concentrations of putrescine. For example, at 1 mM-putrescine, 10 mM-spermidine decreased the uptake rate by 65% and had no effect at 10 mM-putrescine. This contrasts with a 96% reduction in uptake rate at 1 μM -putrescine caused by 1 mM-spermidine. Identical results were obtained with spermine. 1,3-Diaminopropane and cadaverine (1,5-diaminopentane) were also more effective inhibitors at low concentrations of putrescine, although inhibition was less severe than with spermidine and spermine. In view of the apparent competitive effect of the diamines, the data for low putrescine concen-

Fig. 4. Inhibition of putrescine uptake by diamines

Myxamoebae were incubated at pH 7.0 at a density of (a) 2.0×10^6 or (b) 1.2×10^6 cells/ml. Uptake rates were determined 15 min after harvesting in the presence of (a) 1,3-diaminopropane or (b) cadaverine, added at the same time as putrescine. Putrescine concentrations were 1 μM ($\circ$), 2.5 μM ($\bullet$), 10 μM ($\square$) and 25 μM ($\blacksquare$), all at 0.1 $\mu\text{Ci/ml}$.

Table 4. *Effect of bivalent metal ions and ω-aminocarboxylic acids on putrescine uptake*

Myxamoebae were incubated at a density of 5×10^6 cells/ml in either 5 mM-Mes, adjusted to pH 7.0 with KOH solution, or 17 mM-sodium phosphate, pH 7.0, with additions made as indicated. At the time shown $0.29 \mu\text{M}$ - $[^{14}\text{C}]$ putrescine ($62 \mu\text{Ci}/\mu\text{mol}$) was added and the uptake rate determined. Uptake rates are given as percentages of that measured in a control suspension to which no addition had been made.

Addition	Concn. (mM)	Buffer	Time of addition of putrescine (min)	Uptake rate (%)
MgCl ₂	0.2	Mes	15	224
MgCl ₂	20.0	Mes	15	52
CaCl ₂	0.2	Mes	15	250
CaCl ₂	20.0	Mes	15	35
MnCl ₂	0.2	Mes	15	146
MnCl ₂	20.0	Mes	15	13
Glycine	50.0	Phosphate	120	108
β-Alanine	50.0	Phosphate	120	109
4-Aminobutanoic acid	50.0	Phosphate	120	73
5-Aminopentanoic acid	50.0	Phosphate	120	32
6-Aminohexanoic acid	50.0	Phosphate	120	25
8-Amino-octanoic acid	50.0	Phosphate	120	16

Fig. 5. *Effect of time of starvation on putrescine uptake rates* Myxamoebae were incubated at pH 7.0 at a density of 1.65×10^6 cells/ml. Samples were withdrawn periodically; $[^{14}\text{C}]$ putrescine was added and the uptake rate determined. Putrescine concentrations were $1 \mu\text{M}$ (○), 0.1 mM (●) and 2.5 mM (□), all at $0.07 \mu\text{Ci}/\text{ml}$.

trations were plotted by the method of Dixon (1953), but this did not give the intersecting straight lines expected for simple competitive inhibition (Fig. 4). Inhibition by neither spermidine nor diamino propane

was enhanced by preincubation of the cells with inhibitor before addition of $[^{14}\text{C}]$ putrescine. The inhibitory effects were specific for putrescine uptake, since neither spermine nor spermidine inhibited the uptake of 6-amino $[^{14}\text{C}]$ hexanoic acid, measured as described by North & Williams (1978).

High concentrations of bivalent metal ions inhibited putrescine uptake, but at lower concentrations enhanced it (Table 4). The inhibitory effect was non-specific, since the uptake of 6-amino $[^{14}\text{C}]$ hexanoic acid was also inhibited by CaCl_2 and MnCl_2 and to a lesser extent by MgCl_2 .

Putrescine uptake was also inhibited by ω-aminocarboxylic acids, the degree of inhibition being dependent on the chain length of the amino acid (Table 4). A detailed analysis of the inhibitory effect of 6-aminohexanoic acid showed that at low putrescine concentrations the inhibition appeared to be competitive (K_i of 7.5 mM for 6-aminohexanoic acid), although at higher concentrations the inhibition was more complex. Preincubation of the cells with 6-aminohexanoic acid was essential for inhibition. If the amino acid (at 50 mM) was added at the same time as putrescine, the uptake rate, measured over a period of 30 min, was enhanced (1.4 times the control rate), but, when it was added 15, 30, 60 or 120 min before putrescine, the rates were 76, 51, 30 and 25 % of the control rate respectively. The increasing inhibition correlated with the time course for 6-aminohexanoic acid uptake (North & Williams, 1978).

Neither ornithine, the metabolic precursor of putrescine, nor lysine inhibited putrescine uptake.

Effect of starvation on the rate of putrescine uptake

When starved in 17 mM-sodium phosphate buffer, myxamoebae undergo many of the changes charac-

teristic of early development. During this period the ability to take up putrescine altered. At low putrescine concentrations the rate of uptake decreased with time of starvation, but at high concentrations it was less affected (Fig. 5). The loss of uptake ability at low concentrations was due to a decrease in the maximum velocity and not to a change in the K_m .

Discussion

The labelling of myxamoebae with [^{14}C]putrescine is believed to represent uptake into the cells. Putrescine uptake has been investigated in a number of other systems, including *Escherichia coli* (Tabor & Tabor, 1966; Simon *et al.*, 1970; Munro *et al.*, 1974), regenerating rat liver (Dykstra & Herbst, 1965), mouse brain slices (Lajtha & Sershen, 1974), mouse LS cells (McCormick, 1975) and mouse mammary gland (Kano & Oka, 1976), and data from these studies are consistent with the involvement of one or more specific energy-dependent transport systems. Although the results obtained with *D. discoideum* might also fit a model involving a specific putrescine system, we believe such an explanation is unlikely. *D. discoideum* normally feeds by engulfing bacteria, which are digested within the cells to provide nutrients. It would seem unnecessary for the cells to possess specific systems for the transport of nutrients from outside the cells. This would be particularly so with putrescine, since it can be synthesized by the cells (Turner & North, 1977) and is not a growth requirement (Franke & Kessin, 1977). If putrescine enters the cells by a non-specific process, then it is necessary to explain why this process differs from that for other compounds we have investigated, particularly with respect to the involvement of saturable components. Like the uptake of 6-aminohexanoic acid (North & Williams, 1978), putrescine uptake is energy-dependent and probably involves pinocytosis. The most satisfactory explanation for the saturation kinetics, and indeed for all the results presented here, is that putrescine uptake can occur by adsorptive pinocytosis (Silverstein *et al.*, 1977).

In adsorptive pinocytosis material enters the cell bound to the inner aspect of the pinocytotic vesicle membrane, and the uptake rate is determined not only by the size of the vesicle but also by the number, affinity and function of cell-surface sites to which the material binds. Thus the complexity of the putrescine uptake kinetics probably reflects the involvement of a number of different types of binding site, each with a different affinity for putrescine. At a particular extracellular putrescine concentration the properties of the uptake process will depend on the nature of those binding sites available. For example, in the concentration range 1–20 μM a single type of binding site is implicated by the straight-line Lineweaver-Burk plot (Fig. 2a), and the K_m of 9.1 μM is an indica-

tion of the affinity of these sites for putrescine. At millimolar concentrations, however, it is likely that adsorption plays a lesser role as cell-surface sites become saturated. We have observed that at high concentrations uptake is non-saturable, which would be consistent with uptake occurring predominantly in the fluid phase, i.e. fluid-phase pinocytosis.

Differences between the uptake rates for putrescine and 6-aminohexanoic acid (at 1 μM , putrescine is taken up 200 times faster than 6-aminohexanoic acid) also indicate that the processes involved differ. 6-Aminohexanoic acid is probably taken up in the fluid phase, and for the uptake of a given volume of extracellular fluid containing both compounds, considerably more putrescine would be internalized by virtue of its adsorption to the membrane. Similar differences in endocytotic index have been observed in other systems, e.g. between colloidal-gold uptake and the uptake of poly(vinylpyrrolidone) and sucrose by rat peritoneal macrophages (Pratten *et al.*, 1977).

Adsorption of putrescine is probably a fortuitous consequence of the presence of negatively charged groups on the cell surface. Cell electrophoresis studies on *D. discoideum* have shown that there is a decrease in cell-surface charge during starvation (Garrod & Gingell, 1970; Yabuno, 1970; Lee, 1972a,b), due in part to a decrease in the density of the negatively charged groups (Lee, 1972a). Thus the loss of ability to take up putrescine during starvation can be explained as a decrease in the number of adsorption sites. Significantly, uptake at high concentrations of putrescine, which we believe to be less dependent on adsorption, was less affected by starvation.

Other diamines and polyamines are likely to compete with putrescine for the adsorption sites. Variations in the relative affinities for the amines from one type of site to another would explain the complex inhibition kinetics observed. At high putrescine concentrations the amines were less inhibitory, probably because adsorption sites contribute less to the overall uptake. The reason for the apparent competitive inhibition by 6-aminohexanoic acid is not clear at present. Since preincubation with the amino acid was necessary for inhibition, it appears that it is only effective when inside the cells.

Myxamoebae contain 14 mM-putrescine (North & Turner, 1977), and so uptake at all extracellular concentrations used in this study represents transport against an apparent concentration gradient. The ability of the cells to retain putrescine probably results, not only from the rapid uptake rate, but also because the diamine can bind to components such as membranes and nucleic acids inside the cell. The pool of free intracellular putrescine is probably very small. Labelled putrescine was lost from the cells only when they were incubated with high concentrations of extracellular putrescine. The resultant influx

of unlabelled putrescine must have increased the size of the free pool, so that exchange between free unlabelled and bound labelled putrescine occurred. Labelled putrescine released into the free pool could then be excreted or, as demonstrated by the data in Table 3, be metabolized. The excretion of putrescine caused by extracellular diamines and polyamines indicated that these will also exchange with intracellular putrescine.

Although the characteristics of the putrescine-uptake process differ from those of other small molecules, the basic mechanism underlying them all is probably the same, pinocytosis. The rates of putrescine uptake are much faster and the extent of uptake much greater, however, and so measurements of this process may prove to be the most convenient in future uptake studies and may provide information relevant to uptake phenomena in general. Studies on 6-aminohexanoic acid uptake (North & Williams, 1978) have demonstrated a difference in permeability between the axenic strain AX2 used in the present study and all non-axenic and slow-growing axenic strains, which may reflect differences in their abilities to carry out pinocytosis. A detailed comparative study of putrescine uptake would therefore be valuable to determine whether these differences involve other features of the uptake of small molecules.

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