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Studies on the Accumulation of Putrescine and Spermidine in *Escherichia coli*

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Abstract

The rate of accumulation of the polyamines spermidine and putrescine by *E. coli* depended on growth rate. Spermidine accumulation was faster in chemostat cultures with high dilution rates than in those with low dilution rates and was slower in bacteria that had been grown for several generations with either putrescine or spermidine, suggesting that the spermidine-uptake system was repressed by exogenous polyamines. The uptake of spermidine required metabolic energy. Thus accumulation occurred in an energy-starved *unc* strain only upon addition of glucose (or D-lactate to a smaller extent). With glucose present accumulation occurred in an *unc*, *frd* strain under anaerobic conditions, suggesting that ATP drives uptake. However, accumulation was generally sensitive to carbonylcyanide *m*-chlorophenylhydrazone (CCCP), indicating that the proton motive force was involved in uptake. Unlike spermidine, putrescine accumulation was faster in slow-growing than in fast-growing cultures. This may have been due to greater efflux of putrescine at faster growth rates. Accumulation of putrescine was faster following prolonged growth with either putrescine or spermidine, suggesting induction of the putrescine-uptake system by exogenous polyamines. Like spermidine accumulation, putrescine accumulation required metabolic energy. Accumulation was insensitive to CCCP and occurred only when glucose was added to energy-starved *unc* bacteria, suggesting that high-energy bonds may drive the uptake of putrescine.

Introduction

The polyamines putrescine and spermidine occur in relatively high concentrations in procaryotes such as *Escherichia coli* where they are synthesized from ornithine and arginine (Tabor *et al.* 1978). Although spermidine has numerous effects on protein and nucleic acid synthesis *in vitro* (reviewed in Tabor and Tabor 1976), its function(s) *in vivo* has yet to be understood. Polyamines were required for optimal rates of growth of *E. coli* since a mutant that was completely deficient in spermidine biosynthesis (*speD* strain) grew at 75% of the wild-type rate (Tabor *et al.* 1978), and a mutant unable to synthesize putrescine and spermidine [Δ(*speA–speB*), Δ(*speC–glc*), Δ(*speD*)] grew at one-third of the rate of that in amine-supplemented medium (Hafner *et al.* 1979). The requirement of polyamines for growth was made absolute in Δ(*speA–speB*), Δ(*speC*) strains by introduction of *rpsL* mutations (Tabor *et al.* 1981). Putrescine is thought to function in osmoregulation in *E. coli* since in medium to low osmolarity the putrescine content was high, and a sudden increase in external osmolarity caused efflux of putrescine from cells (Munro *et al.* 1972).

E. coli accumulates exogenous polyamines and evidence for separate uptake systems for spermidine and putrescine has been obtained. Thus putrescine accumulation was not inhibited in the presence of 10-fold excess of spermidine; however, spermidine accumulation was inhibited (37%) by a 27-fold excess of putrescine (Tabor and Tabor

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1966). Putrescine accumulation utilized two separate systems in *E. coli*, one of which was present only after prolonged growth in low osmolarity medium (Munro *et al.* 1974).

Accumulation of polyamines appears to require metabolic energy since cultures starved of an energy source or treated with the uncoupler, dinitrophenol, had reduced accumulation of both putrescine and spermidine (Tabor and Tabor 1966). This paper describes studies on some of the factors that influence the accumulation of putrescine and spermidine in *E. coli* and examines the source of energy used for accumulation.

Materials and Methods

Bacterial Strains

The strains used were all *E. coli* K12 derivatives. Strain NSW6 is an *ilv*$^+$, *uncA* transductant from a cross using JP2140 [*his-29(am)*, *trpA9605(am)*, *ilv-1*] as recipient and $P1_{kc}$ phage propagated on AN120 (*uncA401*). Strain NSW28 [*his29(am)*, *trpA9605(am)*, *uncB402*] is an *ilv*$^+$ transductant from a cross using JP2140 and $P1_{kc}$ phage propagated on AN283 (*uncB402*, *argH*, *entA*). Strain AN480 (*uncB401*, *frd-1*, *entA*) was supplied by G. B. Cox (Australian National University, Canberra). Genetic nomenclature is that of Bachmann and Low (1980).

Media and Growth of Bacteria

Bacteria were grown at 37°C in aminoglycoside uptake medium (AUM, Bryan and Van den Elzen 1977) with 0·1% (w/v) casamino acids (Difco) replacing the gelysate peptone. This medium was supplemented with thiamin-HCl (3 μM) and glucose at 20 mM for batch cultures and at 1 mM for chemostat cultures. When appropriate, amino acid supplements were as follows: histidine (0·5 mM), valine (0·7 mM), isoleucine (0·6 mM) and tryptophan (0·2 mM). The osmolarity of AUM was approximately 225 mosmol. Broth cultures (usually 10 or 20 ml) were grown in 125-ml flasks in a reciprocating shaking water-bath at a speed setting of 100 oscillations/min to provide aeration. For continuous cultures, a chemostat of the type described by Muir *et al.* (1984) was used. Briefly the chemostat consisted of a stoppered wide-bore glass tube (volume 20 ml), with ports allowing for inlet of medium and air, and sample collection. The temperature was maintained at 37°C by circulating water through an outer jacket. An outlet tube permitted outflow of culture and gases. Cultures were aerated by bubbling sterile, instrument-grade air (C.I.G., Australia) at 40–50 ml/min. Medium inflow was controlled by a peristaltic pump (Microperpex model 2132, L.K.B.). Glucose was present at 1 mM and casamino acids (Difco, vitamin-free) at 0·1% (w/v). Additional supplements (listed above) were added as required. Under these conditions growth was limited by the available carbon source since increasing the concentration of either glucose or casamino acids resulted in proportionately higher cell densities. The pH of chemostat cultures in steady state at the dilution rates used was the same as that of uninoculated medium.

Cell growth was measured using a Klett–Summerson colorimeter with a blue filter. It was found that a Klett reading of 200 was equivalent to 0·14 mg dry weight of bacteria per millilitre.

Preparation of Non-growing (Resting) Cell Suspensions

Bacteria were harvested from mid-exponential phase batch cultures (Klett reading 150–200), washed once with AUM supplemented with glucose (20 mM) and thiamin-HCl (3 μm) (AUM–glucose) and resuspended to the original cell density in AUM–glucose. For the experiments in which the effect of growth in the presence of putrescine or spermidine on the subsequent accumulation of polyamines was tested, non-growing cell suspensions were prepared from chemostat cultures as described above.

Preparation of Energy-depleted Cell Suspensions

Strain NSW28 (*uncB402*) was depleted of energy reserves as described by Berger (1973). Briefly bacteria were harvested from mid-exponential phase batch cultures, washed twice in AUM then resuspended in AUM which contained dinitrophenol (5 mM). This suspension was incubated at 37°C for 75 min, washed three times with AUM from which the phosphate had been omitted (AUM–Pi), then resuspended in AUM–Pi to a cell density equivalent to a Klett reading of 120. This suspension was divided into two aliquots, to one of which was added sodium arsenate (2 mM). Both aliquots were incubated at 37°C for 20 min prior to beginning accumulation experiments.

Spermidine- and Putrescine-accumulation Experiments

For accumulation experiments using continuous cultures either 50 nmol (9·25 mBq) ml^{-1} of [^{14}C]spermidine or 50 nmol (18·5 mBq) ml^{-1} of [^{3}H]putrescine was first added to the medium supply and then, at the same concentration, to the chemostat at a time coincident with the arrival of medium containing labelled polyamine (zero time). The concentration of putrescine and spermidine used (50 μm) was found to saturate the accumulation systems. Samples (200 μl) were removed from the chemostat at regular time intervals thereafter, and the cells collected on membranes (Amicon, microporous, 0·45 μm pore size). Membranes were rapidly washed with chilled 3% (w/v) NaCl (20 ml), dried and counted in 5 ml of toluene-based scintillation fluid [5 g 2,5-diphenyloxazole (PPO, Packard) and 0·3 g 1,4-bis(4-methyl-5-phenyloxazol-2-yl)benzene (methyl POPOP, Packard) per litre of toluene] using a Packard Tricarb (model 300) liquid scintillation counter. Steady-state growth was not affected by the presence of polyamines since culture optical density readings remained constant for the duration of the accumulation experiments.

Accumulation of putrescine and spermidine in either non-growing cultures or in energy-depleted cell suspensions were carried out using the same concentrations and specific activities of polyamines as in the chemostat-accumulation experiments. Aerobic accumulations were carried out in shake flasks while anaerobic accumulations, using aerobically grown cells, were carried out in tubes that were continually flushed with nitrogen. Accumulation of polyamines in energy-depleted cells of NSW28 (Table 1) was measured by taking samples at regular intervals then calculating the initial rate of accumulation from the slope of the graph at early times in the presence or absence of sodium arsenate (2 mM).

Uptake of Proline and Glutamine

Accumulation of proline and glutamine was measured using a method identical to that used for polyamines. Proline and glutamine were added at 86·8 nmol (37 mBq) ml^{-1} and 68·4 nmol (37 mBq) ml^{-1} respectively.

Efflux of Putrescine and Spermidine

To mid-exponential cultures (Klett reading of 150) was added either 50 nmol (110 mBq) ml^{-1} of [^{3}H]putrescine or 50 nmol (55 mBq) ml^{-1} of [^{14}C]spermidine. Incubation was continued for 20 min after which time the cells were harvested by centrifugation, washed once at 2°C in AUM–glucose and then resuspended in 1 ml of AUM–glucose (at 2°C). Aliquots (0·5 ml) of these suspensions were then diluted using AUM–glucose (4·5 ml) in 125 ml flasks to cell densities corresponding to Klett readings of 150–170. One of the flasks also contained the amino acid supplements for growth (growing culture), the other did not (non-growing culture). The cultures were aerated by shaking at 37°C and samples (200 μl) were removed at regular intervals and quickly filtered. The radioactivity present in the bacteria was determined as described above.

Results

Effect of Growth Rate on Accumulation of Spermidine and Putrescine

Fig. 1*a* shows that the rate of accumulation of spermidine by carbon source-limited chemostat cultures was related to dilution rate. Thus the culture with the highest dilution rate, D ($=0·68\ h^{-1}$) had the fastest rate of accumulation of spermidine. Growth was not essential for accumulation however, since a non-growing culture accumulated spermidine more rapidly than the growing cultures. Putrescine was accumulated by chemostat cultures of *E. coli* less rapidly than spermidine at each dilution rate tested (Fig. 1*b*) and, in contrast to spermidine, accumulation was more rapid in the slower growing cultures ($D = 0·16\ h^{-1}$, $0·33\ h^{-1}$) than in the culture with a higher growth rate ($D = 0·68\ h^{-1}$). The fastest rate of putrescine accumulation occurred in a non-growing culture. The accumulated polyamines were lost immediately upon addition of toluene to the culture. This is consistent with a previous finding (Tabor and Tabor 1966), and indicates that the polyamines were not irreversibly bound to polyacids such as nucleic acid.

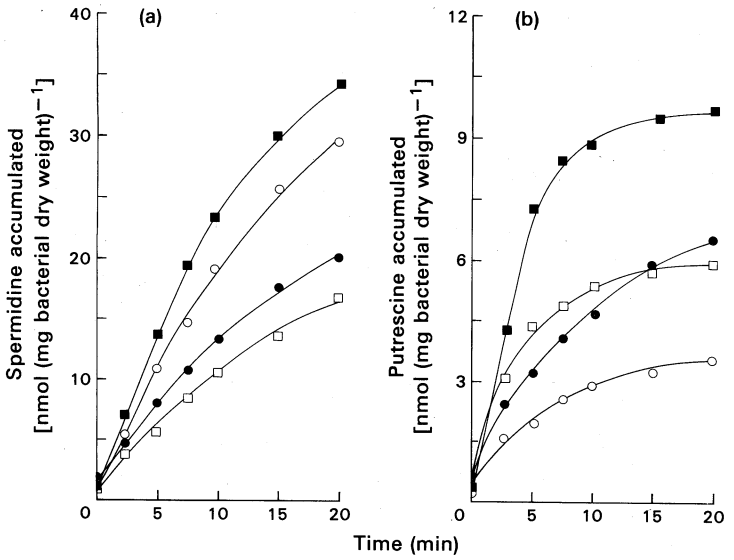

Fig. 1. Effect of dilution rate on spermidine and putrescine accumulation. Strain JP2140 was grown in chemostats as described in Materials and Methods. When steady-state growth was reached accumulation was initiated by addition of either (*a*) 50 nmol (9·25 mBq) ml^{-1} of [^{14}]spermidine or (*b*) 50 nmol (18·5 mBq) ml^{-1} of [^{3}H]putrescine to the chemostat and to the medium reservoir. Samples (200 μl) were removed at regular intervals for counting of radioactivity. Dilution rates: 0·68 h^{-1} (○); 0·33 h^{-1} (●); 0·16 h^{-1} (□). For non-growing cultures (■), cells were collected from mid-exponential phase batch cultures, washed and resuspended in AUM–glucose.

Efflux of Spermidine and Putrescine from Growing and Non-growing Cultures

Efflux of polyamines from growing bacteria, but not from non-growing bacteria, might explain the higher rates of accumulation observed in the latter. Efflux of putrescine

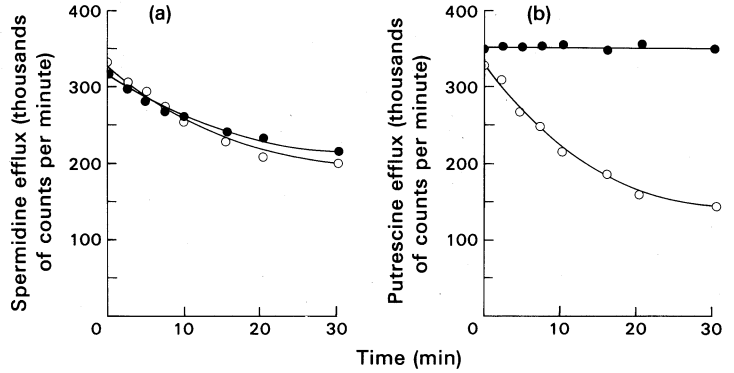

Fig. 2. Efflux of spermidine and putrescine from growing (○) and non-growing (●) bacteria. Mid-exponential phase batch cultures of JP2140 were incubated for 20 min with either 50 nmol (55 mBq) ml^{-1} of [^{14}C]spermidine or 50 nmol (110 mBq) ml^{-1} of [^{3}H]putrescine. Bacteria were harvested, washed at 2°C and resuspended in AUM–glucose at 37°C. Samples (200 μl) were removed at regular intervals and the [^{14}C]spermidine (*a*) and [^{3}H]putrescine (*b*) present in the bacteria was determined.

from *E. coli* has been demonstrated (Simon *et al.* 1970). Measurements of [^{14}C]spermidine or [^{3}H]putrescine retained in cells that had been incubated (i.e. pre-

loaded) with polyamines for 20 min, washed and resuspended in AUM–glucose either supplemented with amino acids (growing culture) or unsupplemented (non-growing culture), indicated that the efflux rate of spermidine was identical for the two cultures over a 30-min period (Fig. 2a). In contrast to spermidine, efflux of putrescine from pre-loaded cells occurred rapidly from a growing batch culture but not at all from a non-growing culture over a 30-min period (Fig. 2*b*).

Accumulation of Spermidine and Putrescine by Cultures Grown in the Presence of Spermidine or Putrescine

Cultures were grown with and without either spermidine (2·5 mM) or putrescine (2·5 mM) in chemostats (D = 0·34 h^{-1}) for approximately 20 generations. Cells were harvested, washed in AUM, resuspended in AUM–glucose and then used for putrescine- and spermidine-accumulation experiments (Fig. 3). Growth with putrescine did not alter

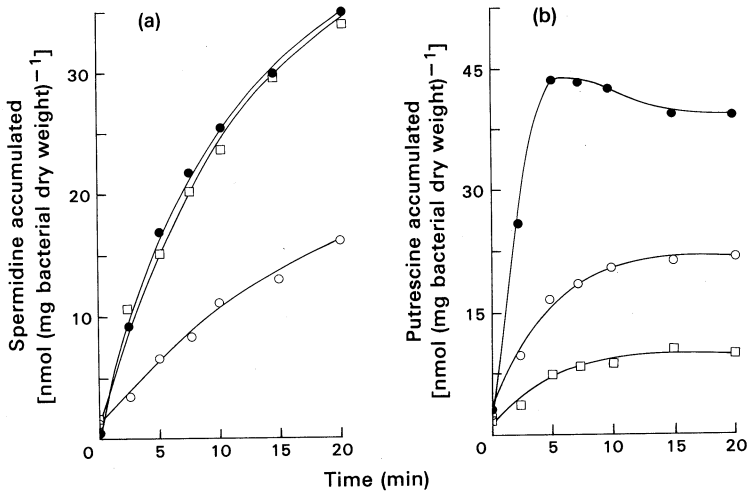

Fig. 3. Effect of prolonged growth with spermidine or putrescine on accumulation of spermidine and putrescine. Strain JP2140 was grown for approximately 20 generations in chemostats (D = 0·34 h^{-1}) in medium either supplemented with spermidine (2·5 mM, ○) or putrescine (2·5 mM, ●); or without polyamine supplementation (□). Bacteria were harvested, washed in AUM, resuspended in AUM–glucose and accumulation of [^{14}C]spermidine (*a*) and [^{3}H]putrescine (*b*) measured.

subsequent accumulation of spermidine (Fig. 3*a*) but caused a marked increase in the rate of putrescine accumulation (Fig. 3*b*). Prolonged growth with spermidine caused a reduction of approximately 50% in spermidine accumulation (Fig. 3*a*), but an approximately twofold increase in the rate of putrescine accumulation (Fig. 3*b*).

Effect of Anaerobiosis and CCCP on Accumulation of Putrescine and Spermidine

Non-growing cultures of strain JP2140 accumulated putrescine (Fig. 4*a*) and spermidine (Fig. 5*a*) under both aerobic and anaerobic conditions. Anaerobic accumulation of both amines was considerably slower than in the aerated cultures. The final levels of accumulation were 80 and 50% of the aerobic value respectively for putrescine and spermidine. Addition of carbonylcyanide *m*-chlorophenylhydrazone

(CCCP; 10 μM) did not inhibit putrescine accumulation in either the aerobic or anaerobic cultures (Fig. 4*a*) but inhibited spermidine accumulation by approximately 30% (Fig. 5*a*). In control experiments, CCCP, at the same concentration, did not inhibit glutamine uptake but reduced proline uptake to less than 10% of that without CCCP.

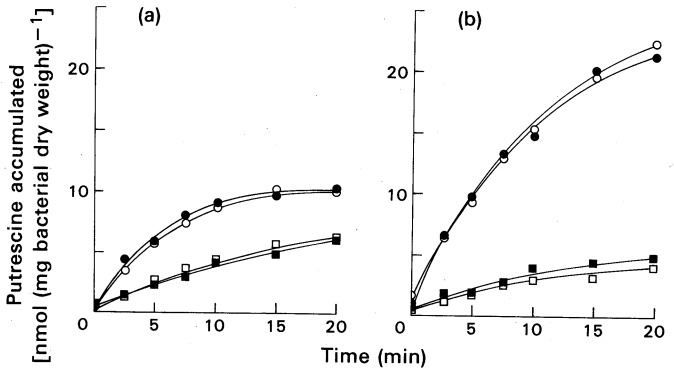

Fig. 4. Accumulation of putrescine by a wild-type and an uncoupled (*unc*) strain under aerobic and anaerobic conditions. Strains JP2140 (*unc*$^+$) (*a*) and NSW6 (*uncA402*) (*b*) were grown aerobically in batch cultures to mid-exponential phase. The bacteria were harvested by centrifugation, washed in AUM and resuspended in AUM–glucose. Accumulation of [^{3}H]putrescine was measured as described above, aerobically with (●) or without (○) CCCP (10 μm); and anaerobically with (■) or without CCCP (□).

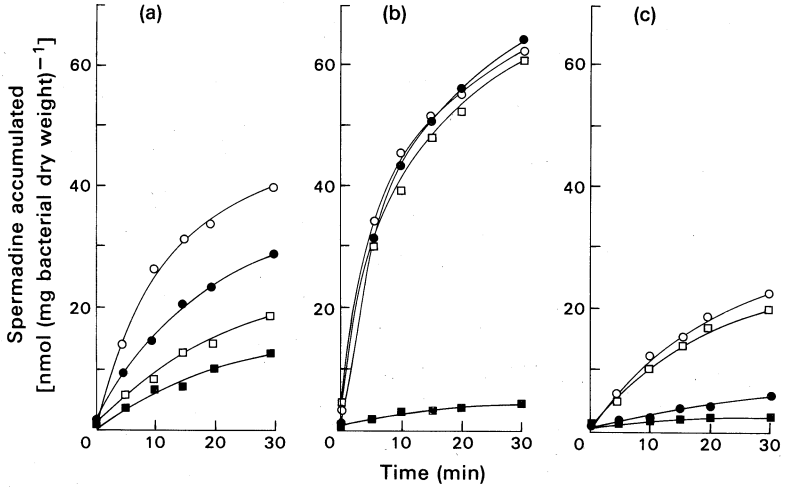

Fig. 5. Accumulation of spermidine by a wild-type and two uncoupled strains. Strains JP2140 (*unc*$^+$) (*a*), NSW6 (*uncA402*) (*b*) and AN480 (*uncB401*, *frd-1*) (*c*) were grown aerobically in batch cultures to mid-exponential phase. The bacteria were harvested, washed in AUM, resuspended in AUM–glucose and tested for accumulation of [^{14}C]spermidine aerobically with (●) and without (○) CCCP (10 μM); anaerobically with (■) and without CCCP (□).

The latter result is consistent with that of Berger (1973) and suggests that, under the conditions used, the proton motive force (Δp) was reduced by CCCP to a sufficiently low enough level to abolish proline transport.

Accumulation of Putrescine and Spermidine in unc *Strains*

Aerated cultures of the *uncA* strain, NSW6, accumulated both putrescine (Fig. 4*b*) and spermidine (Fig. 5*b*) at faster rates than the *unc*$^+$ strain, JP2140. Coupling of respiration to oxidative phosphorylation is therefore not essential for polyamine accumulation.

To try to eliminate any component of accumulation that was driven by the proton motive force we measured accumulation in *unc* strains under anaerobic conditions. Accumulation of putrescine by NSW6 was inhibited in anaerobic conditions but was unaffected by CCCP (Fig. 4*b*). Accumulation of spermidine was unaffected by anaerobiosis (Fig. 5*b*). CCCP inhibited the anaerobic accumulation of spermidine but, in contrast to strains JP2140 and AN480, aerobic accumulation of spermidine by NSW6 was insensitive to CCCP (Figs 5*b*, 5*c*). The reason for the lack of inhibition by CCCP in the *uncA* strain (NSW6) is not understood.

Table 1. Effect of glucose and D-lactate on accumulation of polyamines by energy-depleted bacteria

Energy-depleted cell suspensions of strain NSW28 (*uncB402*) were prepared and accumulation of nutrients measured in the presence or absence of sodium arsenate (2 mM) as described in the Materials and Methods. Values in parenthesis represent the initial rate of accumulation in unstarved non-growing cultures

Accumulation of	Energy source	Initial rate of accumulation[A]	
		Sodium arsenate absent	Sodium arsenate present
Proline	None	2·8	1·7
	D-Lactate	6·2	5·7
	Glucose	2·1 (13·1)	6·9
Glutamine	None	0·08	0·08
	D-Lactate	0·19	0·10
	Glucose	2·6 (5·6)	0·28
Putrescine	None	0·07	0·07
	D-Lactate	0·07	0·07
	Glucose	0·16 (0·99)	0·08
Spermidine	None	0·08	0·04
	D-Lactate	0·11	0·04
	Glucose	0·27 (2·8)	0·30

[A] Expressed as nmoles per minute per milligram dry weight of bacteria.

It was possible that in the *uncA* strain Δp could have been generated under anaerobic conditions by electron transport to either nitrate (which is present in AUM) or fumarate (from glucose). To avoid these possible sources of anaerobic respiration we measured spermidine accumulation in an *uncB*, *frd* strain (AN480) using AUM–glucose in which the sodium nitrate was replaced by sodium chloride. Strain AN480 accumulated spermidine both aerobically and anaerobically, and accumulation under both conditions was largely abolished by CCCP (Fig. 5*c*). The level of aerobic accumulation of spermidine by AN480 was somewhat less than that of JP2140.

Accumulation of Putrescine and Spermidine by Energy-depleted Cultures

To investigate further the source of energy used to drive polyamine uptake, accumulation was measured in cultures that had been depleted of energy reserves by incubation with dinitrophenol (see Materials and Methods). Strain NSW28 (*uncB402*) was used for these experiments as it was easier to deplete this strain of energy reserves than *uncA* or *unc*$^+$ strains. Energy-depleted cells accumulated proline only with addition of either D-lactate or glucose (Table 1). Glutamine accumulation occurred on addition of glucose (but not D-lactate), and addition of sodium arsenate (2 mM) inhibited glucose-dependent glutamine accumulation (Table 1). Accumulation of both putrescine and spermidine was abolished in energy-depleted cultures and, like glutamine uptake, was not restored by addition of D-lactate. Addition of glucose stimulated accumulation of putrescine to approximately 15% of that of unstarved cells and spermidine to approximately 10% of that of the unstarved culture (Table 1). Addition of sodium arsenate (2 mM) abolished the stimulatory effect of glucose on putrescine, but not spermidine, accumulation.

Discussion

In the present study intact cells were used for accumulation experiments. No distinction was made between unmodified polyamines and those that were either bound to nucleic acids or converted to acetylated derivatives. Acetylation of spermidine and putrescine can be prevented by addition of sodium arsenite to the culture (Tabor and Tabor 1966). In experiments with non-growing cultures, accumulation of putrescine and spermidine was not reduced in the presence of sodium arsenite (2 mM). We have assumed that observed differences in accumulation of polyamines in our experiments were not due to conversion of putrescine or spermidine to acetylated derivatives.

Accumulation of spermidine and putrescine were both influenced by growth rate (Fig. 1). Intracellular accumulation of exogenously added metabolites occurs when the rate of uptake of the metabolite exceeds its rate of efflux. In the case of cells pre-loaded with spermidine, efflux rates were slow compared with accumulation rates and were identical for both growing and non-growing cells (Fig. 2*a*). This suggested that it was the rate of uptake of spermidine that was increased at higher growth rates. For putrescine it was not possible to interpret measured accumulation rates in terms of uptake since it was found (Fig. 2*b*) that there was an efflux of putrescine from growing (but not from non-growing) cells. The more rapid accumulation of putrescine by non-growing cells compared to growing cells (and perhaps the more rapid accumulation by slower compared to faster-growing cells) could have resulted from reduced efflux rather than increased uptake.

Previous studies showed that the polyamine content of *E. coli* depended upon growth rate. Thus faster-growing cultures were found to contain higher concentrations of spermidine and putrescine than slower-growing cultures (Morris and Jorstad 1973; Boyle *et al.* 1977). Perhaps the requirement for more spermidine at faster growth rates was related to the intracellular concentration of ribosomes which is higher in faster-growing than in slower-growing bacteria (Maaloe and Kjeldgaard 1966; Harvey 1973). Guanosine 3′,5′-pyrophosphate (ppGpp) has been shown to inhibit ornithine decarboxylase, one of the enzymes of spermidine biosynthesis (Holtta *et al.* 1974). The rapid uptake of spermidine in a non-growing (amino acid-starved) culture (Fig. 1*a*), which would be expected to contain a higher concentration of ppGpp than

a growing culture, may be a response to a reduction in the rate of spermidine biosynthesis.

Both putrescine and spermidine appear to exert regulatory effects on their accumulation systems. Thus the rate of accumulation of putrescine in cells grown for a prolonged period in putrescine-supplemented medium was found to be much greater than for the control culture grown without putrescine (Fig. 3*b*), suggesting that putrescine induced synthesis of the components involved in its uptake. Exogenous spermidine also induced higher rates of putrescine accumulation but to a lesser degree than growth with putrescine. This suggests that the system that regulates putrescine accumulation is sensitive to both putrescine and spermidine, perhaps because of the structural similarities between the two amines. Growth with exogenous spermidine was found to decrease spermidine accumulation (Fig. 3*a*), suggesting that spermidine represses synthesis of the spermidine-uptake system.

Accumulation of polyamines by bacteria required an energy source since an energy-starved *uncB* strain did not accumulate either putrescine or spermidine unless glucose was added (Table 1). In contrast to glucose, D-lactate addition did not stimulate accumulation of putrescine in an energy-starved *uncB* strain suggesting that Δp alone cannot energize accumulation and that high-energy bonds might be involved. This suggestion is consistent with the observation that putrescine accumulation was insensitive to CCCP, an agent that dissipates Δp. However, respiration appeared to be necessary for maximum accumulation rates. It is difficult to understand how respiration was involved in supplying energy other than through generation of Δp.

Spermidine accumulation was sensitive to oxygen tension in the *unc*$^+$ strain (JP2140) but insensitive in the two *unc* mutants, NSW6 and AN480. We do not know the means through which the activity of adenosinetriphosphatase affects the sensitivity of spermidine accumulation to oxygen. The observation that respiration is not essential for spermidine accumulation was confirmed by the anaerobic experiment with the *uncB*, *frd* strain, AN480 (Fig. 5*c*). In this experiment electron transport was absent since the electron acceptors, oxygen and nitrate, were not available, and fumarate reduction was blocked by the *frd* mutation. Thus Δp cannot be generated from glucose either by respiration or, because of the *uncB* mutation, by hydrolysis of ATP. Although proline uptake was absent under these conditions, the kinetics of spermidine accumulation was the same as for the aerobic culture (Fig. 5*c*), suggesting that accumulation of spermidine can be energized by ATP derived from glucose by substrate-level phosphorylation. This tentative conclusion was supported by the observation that addition of glucose stimulated spermidine accumulation in an energy-depleted *uncB* strain to a greater extent than D-lactate (Table 1). However, the level of glucose-stimulated accumulation was only 10% that of an unstarved *uncB* culture and accumulation was not abolished when ATP formation was blocked by addition of sodium arsenate. Moreover, accumulation of spermidine was sensitive to CCCP. These results do not permit identification of the source of energy for spermidine accumulation in terms of either high-energy bonds or the proton motive force, and suggest a more complex energy requirement than that for either proline or glutamate.

Acknowledgments

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References

Bachmann, B. J., and Low, K. B. (1980). Linkage map of *Escherichia coli* K12, edition 6. *Microbiol. Rev.* **44**, 1–56.

Berger, E. A. (1973). Different mechanisms of energy coupling for the active transport of proline and glutamine in *Escherichia coli*. *Proc. Natl Acad. Sci. U.S.A.* **70**, 1514–18.

Boyle, S. M., MacIntyre, F., and Sells, B. H. (1977). Polyamine levels in *Escherichia coli* during nutritional shiftup and exponential growth. *Biochim. Biophys. Acta* **477**, 221–7.

Bryan, L. E., and Van den Elzen, H. M. (1977). Effects of membrane-energy mutations and cations on streptomycin and gentamicin accumulation by bacteria: a model for entry of streptomycin and gentamicin in susceptible and resistant bacteria. *Antimicrob. Agents Chemother.* **12**, 163–77.

Hafner, E. W., Tabor, C. W., and Tabor, H. (1979). Mutants of *Escherichia coli* that do not contain 1,4-diaminobutane (putrescine) or spermidine. *J. Biol. Chem.* **254**, 12419–26.

Harvey, R. J. (1973). Fraction of ribosomes synthesizing protein as a function of specific growth rate. *J. Bacteriol.* **114**, 287–93.

Holtta, E., Janne, J., and Pispa, J. (1974). The regulation of polyamine synthesis during the stringent control in *Escherichia coli*. *Biochem. Biophys. Res. Commun.* **59**, 1104–11.

Maaloe, O., and Kjeldgaard, N. O. (1966). 'Control of Macromolecular Synthesis.' (Benjamin: New York.)

Morris, D. R., and Jorstad, C. M. (1973). Growth and macromolecular composition of a mutant of *Escherichia coli* during polyamine limitation. *J. Bacteriol.* **113**, 271–7.

Muir, M. E., Van Heeswyck, R. S., and Wallace, B. J. (1984). Effect of growth rate on streptomycin accumulation by *Escherichia coli* and *Bacillus megaterium*. *J. Gen. Microbiol.* **130**, 2015–22.

Munro, G. F., Bell, C. A., and Lederman, M. (1974). Multiple transport components for putrescine in *Escherichia coli*. *J. Bacteriol.* **118**, 952–63.

Munro, G. F., Hercules, K., Morgan, J., and Sauerbier, W. (1972). Dependence of the putrescine content of *Escherichia coli* on the osmotic strength of the medium. *J. Biol. Chem.* **247**, 1272–80.

Simon, E. J., Schapira, L., and Wurster, N. (1970). Effect of levorphanol on putrescine transport in *Escherichia coli*. *Molec. Pharmacol.* **6**, 577–87.

Tabor, C. W., and Tabor, H. (1966). Transport systems for 1,4-diaminobutane, spermidine, and spermine in *Escherichia coli*. *J. Biol. Chem.* **241**, 3714–23.

Tabor, C. W., and Tabor, H. (1976). 1,4-Diaminobutane (putrescine) spermidine, and spermine. *Ann. Rev. Biochem.* **45**, 285–306.

Tabor, H., Tabor, C. W., Cohn, M. S., and Hafner, E. W. (1981). Streptomycin resistance (*rpsL*) produces an absolute requirement for polyamines for growth of an *Escherichia coli* strain unable to synthesize putrescine and spermidine [Δ(*speA-speB*) Δ*speC*]. *J. Bacteriol.* **147**, 702–4.

Tabor, C. W., Tabor, H., and Hafner, E. W. (1978). *Escherichia coli* mutants completely deficient in adenosylmethionine decarboxylase and in spermidine biosynthesis. *J. Biol. Chem.* **253**, 3671–6.

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