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Transport of Adenine, Hypoxanthine and Uracil into *Escherichia coli*

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Uptake of adenine, hypoxanthine and uracil by an *uncA* strain of *Escherichia coli* is inhibited by uncouplers or when phosphate in the medium is replaced by less than 1 mM-arsenate, indicating a need for both a protonmotive force and phosphorylated metabolites. The rate of uptake of adenine or hypoxanthine was not markedly affected by a genetic deficiency of purine nucleoside phosphorylase. In two mutants with undetected adenine phosphoribosyltransferase, the rate of adenine uptake was about 30% of that in their parent strain, and evidence was obtained to confirm that adenine had then been utilized via purine nucleoside phosphorylase. In a strain deficient in both enzymes adenine uptake was about 1% of that shown by wild-type strains. Uptake of hypoxanthine was similarly limited in a strain lacking purine nucleoside phosphorylase, hypoxanthine phosphoribosyltransferase and guanine phosphoribosyltransferase. Deficiency of uracil phosphoribosyltransferase severely limits uracil uptake, but the defect can be circumvented by addition of inosine, which presumably provides ribose 1-phosphate for reversal of uridine phosphorylase. The results indicate that there are porter systems for adenine, hypoxanthine and uracil dependent on a protonmotive force and facilitated by intracellular metabolism of the free bases.

Nucleic acid bases and their ribosides are readily converted into nucleotides by *Escherichia coli*, only a small proportion being found as free base or nucleoside inside the cells. Hochstadt-Ozer & Stadtman (1971*a*,*b*,*c*) concluded that adenine is taken up by a group translocation involving adenine phosphoribosyltransferase (EC 2.4.2.7). Hochstadt (1974) has reviewed evidence for this and similar group-translocation processes, including reports that uracil is not taken up by mutants lacking uracil phosphoribosyltransferase (EC 2.4.2.9) (Beck & Ingraham, 1971; Pierard *et al.*, 1972). Evidence against a simple group-translocation process was reported by Roy-Burman & Visser (1975), who found K_m values for uptake to be much smaller than those reported for purified phosphoribosyltransferases, and claimed that several purines and pyrimidines, including adenine and uracil, were accumulated as free bases against a concentration gradient. It is, however, difficult to be sure that the small amounts of free intracellular bases were not formed by degradation of nucleotides during the extraction procedure. Kocharian *et al.* (1975) have given evidence that adenine phosphoribosyltransferase is not needed for adenine utilization.

In the present paper, I aim to clarify the relation between energy coupling and metabolic conversions in the transport of adenine, hypoxanthine and uracil.

Materials and Methods

Bacteria

In addition to *E. coli* B, several *E. coli* K12 strains were used. Strain A103c (Ymel,*ato*,*fadR*^{c},*lacI*,*metE*,-*rha*,*uncA*) and its parent strain A1002 (isogenic except for being *ilv*,*uncA*$^+$) were both described by Schairer & Haddock (1972); strain PA309 (*thr*,*leu*,-*thi*,*his*,*trp*,*argH*,*lacY*,*gal*,*malA*,*xyl*,*ara*,*mtl*,*tonA*,*str*; cf. Bachman, 1972) was from Professor H. L. Kornberg; strains NE553 and NE571 were mutants of strain PA309 resistant to 2,6-diaminopurine and found to be deficient in adenine phosphoribosyltransferase (*apt* genotype; Jochimsen *et al.*, 1975). They arose in separate treatments of growing bacteria with 2 μg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine/ml as described by Miller (1972). Strain 4K3 (*thr*,*leu*,*thi*,*ser*,*ars*,*upp*; Beacham & Pritchard, 1971) had been derived from *E. coli* C600 via strain 4K (Glover, 1970); strains SØ609 (*ara*,*Δpro-gpt-lac*,*thi*,-*hpt*,*pup*,*purH* or *purJ*,*strA*), SØ199 (*metB*,*strA*,*purE*) and its derivatives SØ312 (also *pup*) and SØ446 (also *pup*,*apt*) are described by Jochimsen *et al.* (1975). Relevant phenotypes were routinely checked by appropriate growth tests.

Growth

Media contained glucose and inorganic salts (Thomas *et al.*, 1970) supplemented with thiamin hydrochloride (1 mg/litre) and trace metal salts (Burton, 1976). Required L-amino acids or purines were added at 10 mg/litre. L-Histidine hydrochloride was also added for all purine-requiring strains. Both L-isoleucine and L-valine were added for both strains A1002 and A103c. Acid-hydrolysed casein (0.1%; Oxoid, London SE1 9HF, U.K.) was also

added for strains NE571 and 4K3, since growth was otherwise poor. Adenosine (50mg/litre) was used as the purine source for strain SØ609, hypoxanthine for strain SØ446 and adenine for strains SØ199 and 312. Bacteria were grown at 37°C with aeration to 2×10^8–4×10^8 cells/ml, centrifuged (4500*g* for 10min) at 4°C, suspended in the original volume of phosphate/saline (24mM-Na_2HPO_4, 11mM-KH_2PO_4, 0.1M-NaCl, 83μM-$MgSO_4$, pH7.3), centrifuged again and suspended in the phosphate/saline at about 2×10^9–5×10^9 cells/ml. Phosphates were omitted from the washing solution when the effects of arsenate were to be examined. Cell concentrations were calculated turbidimetrically, on the basis of a calibration against viable cell count with *E. coli* B (1.9×10^9 dry cells weighed 1.0mg).

Measurement of uptake by filtration and washing

Washed bacteria stored for less than 2h at 0–4°C were preincubated for 15min with the energy source (usually glucose) and chloramphenicol in 1–2ml of phosphate/saline shaken at 37°C in a 25ml conical flask. If further additions were greater than 100μl, they were dissolved in phosphate/saline. Measured samples (0.25–0.35ml) were removed directly on to a 21mm-diameter cellulose acetate filter (Oxoid) lying on a stainless-steel mesh support connected to suction. The filters were washed quickly with 3×1ml of phosphate/saline warmed to 37°C to avoid cold shock and followed by 0.5ml of ethanol to speed drying. Radioactivity was measured with efficiencies of 12% for ^3H and 66% for ^{14}C by liquid-scintillation counting (see Burton, 1976).

Measurement of uptake without filtration

This procedure was used to eliminate any losses by leakage during washing. Approx. 2ml of incubation mixture containing 1–2mg dry wt. of bacteria was filtered as above but by using a filter tower and with no washing: 30s after filtration appeared to be complete, the moist filter was removed from the suction apparatus and immediately weighed. Evaporation was negligible, and so the volume of water on the filter (about 25μl) could be found after subtraction of the dry weight of the filter plus bacteria. The filters were dried by air at room temperature. To obviate any colour quenching when carbonyl cyanide *m*-chlorophenylhydrazone was present, ^{14}C-labelled precursors were used and the ^{14}C was measured at 25% efficiency in a solid-sample counter. Comparison of the radioactivity found per mg of water that had been on the moist filter with the concentration of radioactivity in the medium indicated the extent of intracellular concentration of radioactivity.

Incorporation into acid-insoluble material

Samples were taken into at least 10vol. of 0.5M-$HClO_4$ at 0°C, filtered on Oxoid filters and washed with 3×3ml of ice-cold 5% trichloroacetic acid, followed by 1ml of ethanol, before measurement of the radioactivity.

Intracellular fates of ^{14}C-labelled compounds

Washed bacteria (approx. 4×10^8 cells) were incubated in 4.4ml of phosphate/saline containing 52mM-glucose and 0.36mM-chloramphenicol. After 15min, 0.2μCi of ^{14}C-labelled substrate was added to give a final concentration of approx. 0.8μM. After incubation for 0.5–3min, the bacteria were collected on an Oxoid filter and washed with 3×1ml of phosphate/saline at 37°C. The filter was then immersed in 0.3ml of 4M-formic acid at 0°C to extract nucleotides (Bagnara & Finch, 1974) and stored at −70°C until required. The interval was about 30s between pouring the bacteria on to the filter and immersion in formic acid. For [^{14}C]adenine or [^{14}C]hypoxanthine portions (50–100μl) of the extract were applied after centrifugation (6000*g* for 20min) as a 1.5cm band on to duplicate papers (0.55m×0.4m) and electrophoresed under 'white spirit' for about 40min with 2kV applied voltage by using 7% (v/v) formic acid for one paper and 5% (v/v) pyridine/0.5% acetic acid (pH6.5) for the other. Free adenine was also separated by paper chromatography in ethylacetate/98% (w/w) formic acid/water (7:2:1, by vol.). Descending paper chromatography in propan-2-ol/conc. aq. NH_3 (sp.gr. 0.880)/water (14:3:3 by vol.) was used for [^{14}C]glycine. Strips were examined in a paper-strip chromatogram scanner and the positions of the ^{14}C peaks compared with the positions of appropriate markers run on the same sheet of paper. Quantitativ ecomparisons were based on liquid-scintillation counting of the appropriate areas of paper.

Phosphoribosyltransferases

The adenine enzyme was assayed as described by Hochstadt-Ozer & Stadtman (1971*a*), except that AMP and adenine were separated by descending paper chromatography in 1M-ammonium acetate. The uracil enzyme was assayed similarly except that 10mM-GTP was added (Molloy & Finch, 1969) and the chromatography was on DEAE-paper developed by 0.01M-Tris/HCl, pH8.0. For testing the inhibition of the adenine enzyme by hypoxanthine, the concentrations of adenine, 5-phosphoribosyl 1-pyrophosphate and extract protein were lowered to 0.18μM, 0.4mM and 11μg/ml respectively.

Other materials

Radiochemicals were from The Radiochemical Centre, Amersham, Bucks., U.K. 3,5,3′,4′-Tetra-

chlorosalicylanilide was very kindly given by Dr. W. A. Hamilton, University of Aberdeen.

Results

Concentration-dependence for adenine uptake

Initially, adenine uptake was studied because of the extensive work of Hochstadt-Ozer & Stadtman (1971*a*,*b*,*c*) with this base. They reported K_m values of 15–20 μM for adenine uptake by non-growing *E. coli* or by membrane vesicles and stressed that these K_m values agreed with those found for the purified adenine phosphoribosyltransferase. However, Roy-Burman & Visser (1975) reported much lower K_m values for the uptake by growing *E. coli* (e.g. 0.12 μM for strain B), and I have approximately confirmed this particular value. Since regulatory controls in growing bacteria (cf. Ames, 1964) might cause erroneously low K_m values, growth was prevented in further experiments by the presence of chloramphenicol and the absence of a readily utilizable nitrogen source. As shown in Fig. 1, the total uptake process is virtually saturated even at the lowest concentration of adenine used (0.78 μM). Quite similar results were obtained with bacteria that had been washed and stored for 48 h at 4°C. The high K_m values of Hochstadt-Ozer & Stadtman (1971*c*) are evidently not due to the lack of growth or to the aging of the bacteria. Because the rate of uptake may fall rapidly after the first 2 min, the duration of the uptake measurements was kept below 3 min by changing the bacterial concentration in parallel with the adenine concentration. The rates of uptake observed in Fig. 1 are at least as great as those in growing cultures. Approx. 87% of the radioactivity in the washed cells was soluble in 0.5 M-$HClO_4$ at 0°C, at least 75% being acid-soluble nucleotides.

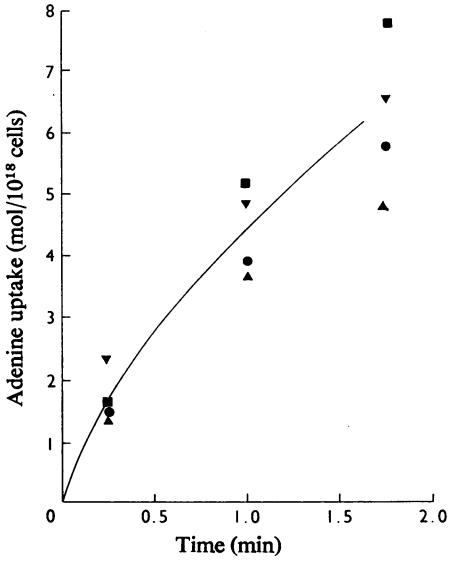

Fig. 1. *Uptake of adenine by washed suspensions of E. coli B at different concentrations of adenine*

Uptake was measured by filtration and rapid washing from an incubation medium containing 4.9 mM-glucose and 0.33 mM-chloramphenicol, but no source of nitrogen. After preincubation for 15 min at 37°C, [2-^{3}H]adenine of different specific radioactivities was added at zero time to give 110 μCi/litre. Each point was obtained by totalling the ^{3}H taken up by three different mixtures containing different amounts of bacteria and dividing by the total number of bacteria. (There was reasonable proportionality between uptake and number of bacteria.) ●, 0.78 μM-Adenine, 0.3×10^8–1.2×10^8 cells/ml; ▲, 1.95 μM-adenine, 1.2×10^8–4.7×10^8 cells/ml; ■, 3.9 μM-adenine, 1.2×10^8–6×10^8 cells/ml; ▼, 19.5 μM-adenine, 3.5×10^8–12×10^8 cells/ml.

Energy coupling in the uptake of adenine, hypoxanthine and uracil

Adenine uptake occurs normally in an *uncA* strain, A103c (Schairer & Haddock, 1972), which has no adenosine triphosphatase to interconvert ATP energy and the protonmotive force. The uptake is, however, more sensitive to carbonyl cyanide *m*-chlorophenylhydrazone than it is in the isogenic *uncA*$^+$ strain A1002 (Fig. 2). The sensitivity to uncouplers is not appreciably affected by increasing the concentration of adenine 100-fold (Fig. 3). Anaerobiosis also inhibits uptake in both strains. Carbonyl cyanide *m*-chlorophenylhydrazone and other agents with uncoupling activity have quite similar effects on the uptake of adenine, hypoxanthine, uracil and glycine in strain A103c (Table 1), but they have negligible effects on the rate of O_2 consumption. The effects of carbonyl cyanide *m*-chlorophenylhydrazone were not reversed by dithiothreitol or mercaptoethanol, showing that it was not inhibiting by virtue of its reported action on thiol groups (Kaback *et al.*, 1974). Since glycine transport is linked to proton movements (Collins *et al.*, 1976), the findings imply that uptake of adenine, hypoxanthine or uracil needs a protonmotive force independently of any need for ATP or other energy-rich metabolites.

Replacement of phosphate in the incubation medium by arsenate severely inhibits the uptake of adenine, hypoxanthine and uracil, but not the uptake of glycine (Fig. 4), originally shown by Berger & Heppel (1974) to be insensitive to arsenate. As might be expected in the presence of chloramphenicol, the [^{14}C]glycine was found to remain as the free amino acid, in contrast with the nucleic acid bases, which were found predominantly as nucleotides or nucleic acids.

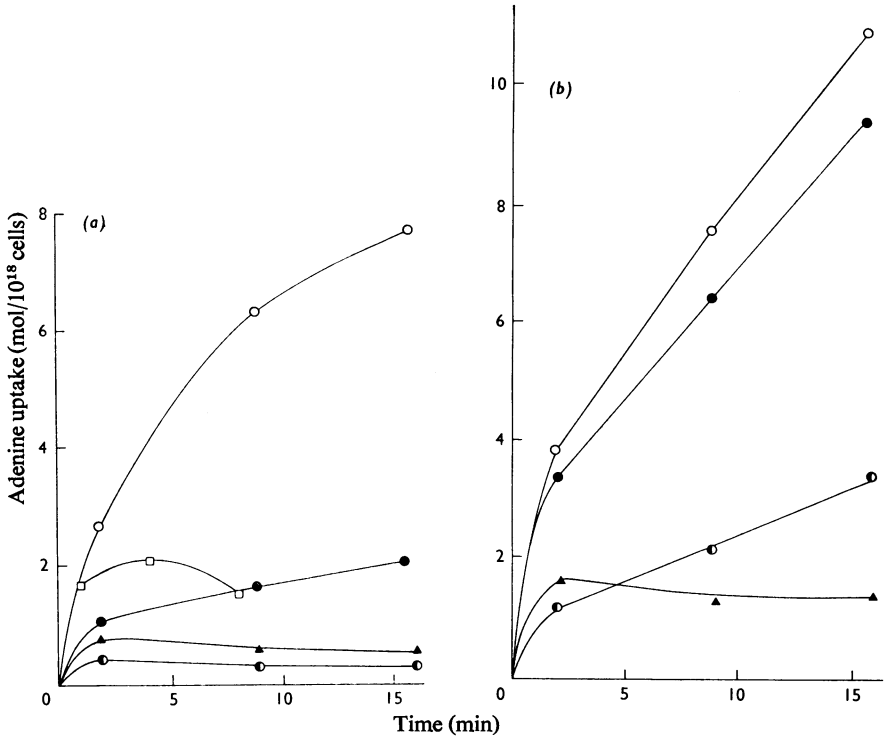

Fig. 2. *Effects of carbonyl cyanide m-chlorophenylhydrazone and requirement for O_2 on adenine uptake in uncA and uncA$^+$ strains*

Conditions were as for Fig. 1, but 4.7mM-glucose, 0.32mM-chloramphenicol, 5×10^7 cells/ml and 0.78μM-[2-^{3}H]adenine were used, with: ○, no further addition; ▲, 42μM-carbonyl cyanide *m*-chlorophenylhydrazone at −15min; ●, 10.5μM-carbonyl cyanide *m*-chlorophenylhydrazone at −15min; ◐, 42μM-carbonyl cyanide *m*-chlorophenylhydrazone at zero time; □, at −5min flask contents were transferred to a test tube at 37°C and bubbled with O_2-free N_2. (*a*) Strain A103c (*uncA*). (*b*) Strain A1002 (*uncA*$^+$).

Table 1. *Effects of agents with uncoupling activity on uptake of adenine, hypoxanthine, uracil and glycine in an uncA strain*

Conditions were as for Fig. 4. Values are uptake measured 2min after addition of the transported substrate expressed as percentages of that without uncoupler. Uncouplers were added to give the final concentrations shown 1min before the substrate: 1mM-1,4-DL-dithiothreitol or 1mM-mercaptoethanol was added 0.5min before substrate where indicated. The thiol compounds had no significant effect by themselves.

Uncoupler	Uncoupler concn. (mM)	Percentage uptake			
		Adenine	Hypoxanthine	Uracil	Glycine
Carbonyl cyanide *m*-chlorophenylhydrazone	0.009	50	18	19	14
Carbonyl cyanide *m*-chlorophenylhydrazone	0.044	24	12	8	9
Carbonyl cyanide *m*-chlorophenylhydrazone and dithiothreitol	0.044	24	8	11	12
Carbonyl cyanide *m*-chlorophenylhydrazone and mercaptoethanol	0.044	24	10	—	—
Tetrachlorosalicylanilide	0.018	—	25	51	31
Tetrachlorosalicylanilide	0.036	15	10	14	14
2,4-Dinitrophenol	0.88	66	85	73	73
2,4-Dinitrophenol	1.76	52	70	48	51

Since arsenate inhibits the formation of phosphate esters by phosphorolysis and other substrate-linked phosphorylation reactions, there may be a coupling between transport and intracellular metabolism of the bases by reaction with ribose 1-phosphate or 5-phosphoribosyl 1-pyrophosphate. This suggestion

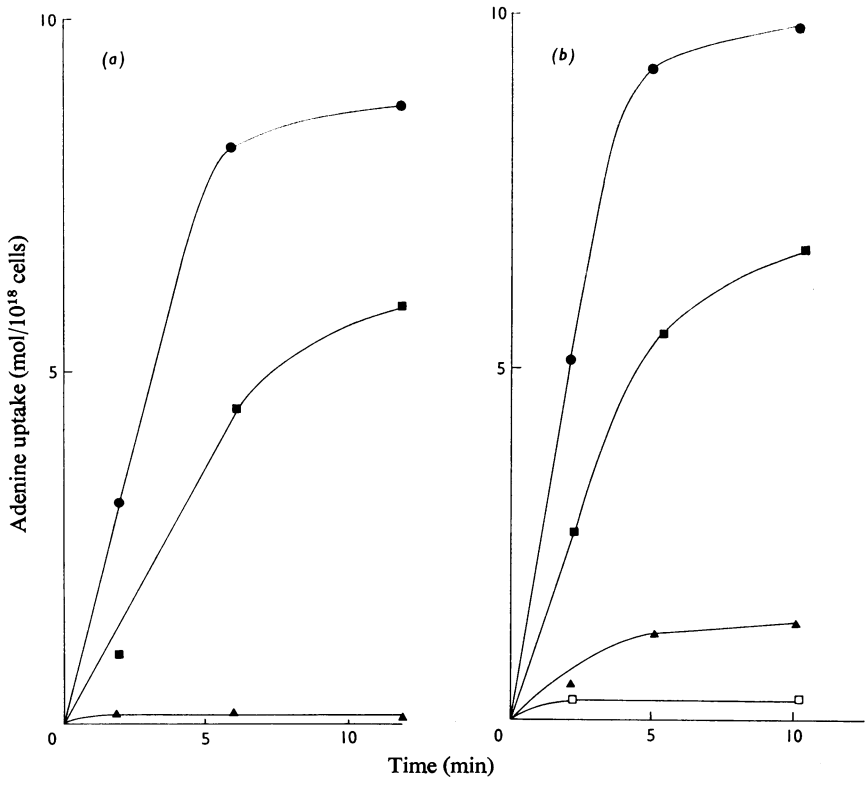

Fig. 3. *Uptake of adenine at different concentrations and the effects of uncouplers in an uncA strain (A103c)*
Details were as for Fig. 2, except final concentrations were (*a*) 4.5×10^7 bacteria/ml and 0.70 μM-[2-^{3}H]adenine (150 Ci/mol) or (*b*) 4.5×10^8 bacteria/ml and 73 μM-[2-^{3}H]adenine (1.44 Ci/mol). ●, No further addition; ▲, 42 μM-carbonyl cyanide *m*-chlorophenylhydrazone added at −15 min; ■, 84 μM-2,4-dinitrophenol added at −15 min; □, no glucose.

is consistent with the ability of uridine to support adenine uptake better than lactate does in the absence of glucose (Fig. 5). Uridine and lactate give similar rates of O_2 consumption (38% of that given by glucose) and are thus expected to generate similar protonmotive forces.

Uptake of adenine and hypoxanthine in a pup mutant

Both purines are taken up by the *pup* strain SØ312 at rates closely similar to those shown by the *pup*$^+$ parent, strain SØ199. For this comparison, the same medium, which contained adenine, hypoxanthine and histidine was used to grow both strains, and uptake was measured as for Fig. 2. Most, if not all, of the transport of adenine and hypoxanthine does not therefore depend on purine nucleotide phosphorylase.

Role of adenine phosphoribosyltransferase in adenine uptake

By using the method of Kalle & Gots (1961), who obtained *apt* mutants of *Salmonella typhimurium* by selection for resistance to 2,6-diaminopurine, similar mutants of *E. coli* resistant to 2,6-diaminopurine at 500 μg/ml were isolated. No adenine phosphoribosyltransferase activity was found in strains NE553 and NE571, and adenine was taken up much more slowly than by the parent strain, PA309 (Fig. 6). Also, in the *apt* strains less than 7% of the ^{3}H taken up was found in acid-insoluble material, compared with 20–50% in strain PA309. The large fraction of macromolecular synthesis in the parent strain is a consequence of the richer growth medium that was used for this particular experiment because of the poor growth of strain NE571 on simpler media. The smaller incorporation of ^{3}H into macromolecules by the *apt* strains suggested that the [^{3}H]adenine might not have been converted into adenine nucleotides in the conditions of the uptake measurements.

In further experiments, strains PA309 and NE553 were grown on minimal medium supplemented with the required amino acids, washed and supplied with [8-^{14}C]adenine under the usual conditions for uptake measurement as in Fig. 1, except that L-histidine was

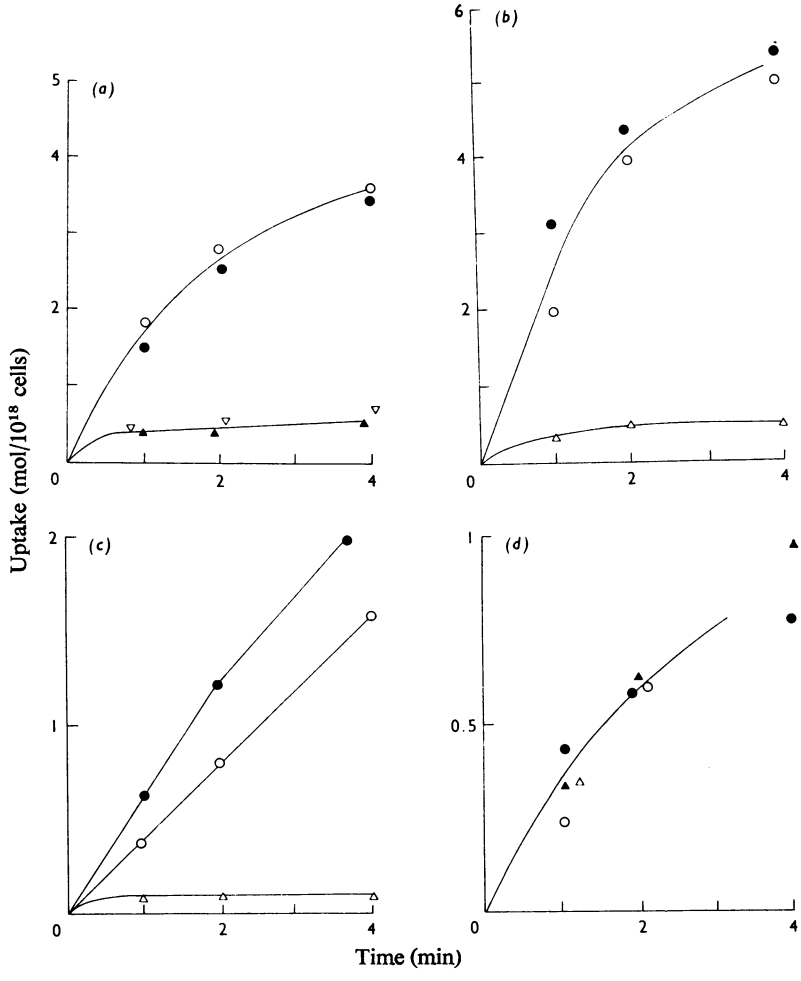

Fig. 4. *Inhibition of uptake of adenine, hypoxanthine and uracil by arsenate*

Strain A103c was washed and re-suspended in Tris/saline (0.1 M-NaCl, 0.058 M-Tris and 0.65 mM-$MgSO_4$ adjusted to pH 7.3 by adding HCl) at 1.7×10^8 cells/ml. Chloramphenicol and glucose were added as for Fig. 1. Additions were made as follows, 0.5 min before radioactive substrate: ○, none; ●, 4.6 mM-Na_2HPO_4 (final concentration) ▲, 4.6 mM-Na_2HAsO_4; △, 0.92 mM-Na_2HAsO_4; ▽, 0.23 mM-Na_2HAsO_4. (*a*) [2-^{3}H]Adenine (0.77 μM, 115 μCi/litre). (*b*) [2-^{14}C]Uracil (0.85 μM, 12 μCi/litre). (*c*) [8-^{14}C]Hypoxanthine (0.91 μM, 47 μCi/litre). (*d*) [1-^{14}C]Glycine (0.91 μM, 51 μCi/litre).

added to prevent histidine biosynthesis, which could conceivably generate ^{14}C-labelled 5-amino-4-imidazolecarboxamide ribonucleotide and hence [^{14}C]-IMP via [^{14}C]ATP. After uptake of [^{14}C]adenine for 1 or 2 min, the mixtures were rapidly filtered, the bacteria washed and nucleotides extracted with 4 M-formic acid. Strain NE553 gave essentially one radioactive component, which migrated on electrophoresis as 5′-IMP. The parent, strain PA309, gave little [^{14}C]IMP, most ^{14}C being in the areas occupied by AMP, NAD and ADP. The deamination of adenine in strain NE553 could only have occurred at the nucleoside level (Koch & Vallee, 1959; Jochimsen *et al.*, 1975), and thus the adenine must have been first converted into adenosine, presumably by purine nucleoside phosphorylase (EC 2.4.2.1). The uptake could not have been linked to a hypothetical latent adenine phosphoribosyltransferase, since the ^{14}C would then have been found mainly in adenine nucleotides.

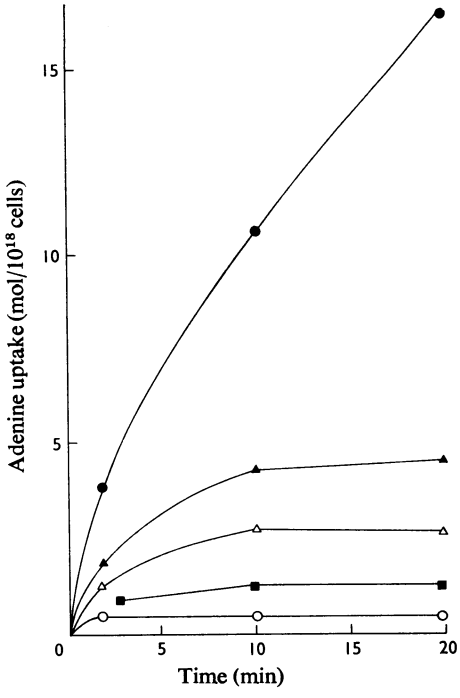

Fig. 5. *Abilities of certain carbon sources to support adenine uptake by the uncA strain, A103c*
Details were as for Fig. 2, except that 3.2×10^7 cells/ml were used, and the carbon sources added at −15 min were: ○, none; ●, 4.9 mM-glucose; ■, 22 mM-DL-lactate; △, 8.8 mM-uridine; ▲, 22 mM-DL-lactate plus 8.8 mM-uridine.

Uptake of adenine and hypoxanthine in strains lacking purine nucleoside phosphorylase and phosphoribosyltransferase enzymes

Uptake and metabolism of the two purines are very much diminished in *pup* strains that are also deficient in the appropriate phosphoribosyltransferase activity. Because of their deficiency in the metabolism of adenine or hypoxanthine, uptake was measured without any washing in case the free purines were leached out of the cells.

With strain SØ446 (*apt,pup*), [8-^{14}C]adenine was incorporated into $HClO_4$-insoluble material at about 0.2% of the rate for wild-type bacteria. The total uptake in strain SØ446 was considerably greater than that into acid-insoluble material, especially in the first 90s, but little ^{14}C was lost on washing. Carbonyl cyanide *m*-chlorophenylhydrazone inhibited the uptake strongly (Fig. 7). Between 15 and 20% of the ^{14}C extracted from washed bacteria by 4 M-formic acid was found as free adenine after uptake for 1 or 3 min. Adenine is readily taken up by

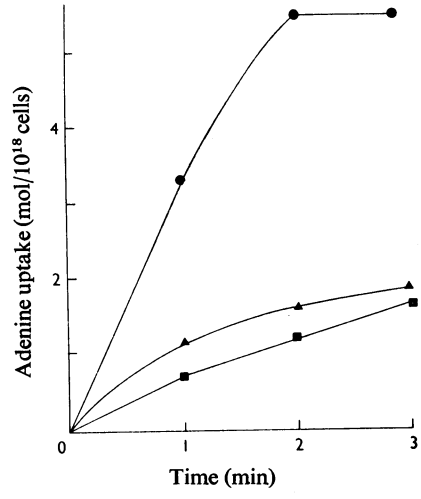

Fig. 6. *Adenine uptake in apt strains*
Details were as for Fig. 1 except that 0.66 μM-adenine (200 μCi/litre) was used. Strains: ●, PA309; ▲, NE553; ■, NE571.

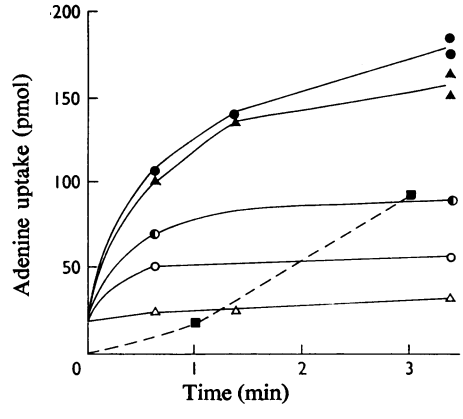

Fig. 7. *Uptake of adenine by strain SØ446 (apt,pup)*
The experiment used 1.3×10^9 cells in 2.22 ml total volume, as for Fig. 1 with [8-^{14}C]adenine (0.78 μM, 58 Ci/mol) added after the preincubation. Uptake measured after washing the filtered bacteria (▲) or with 150 μM-carbonyl cyanide *m*-chlorophenylhydrazone (△). Uptake measured without washing, but corrected for ^{14}C in residual medium on the filters (●) or (◐) with 45 μM-carbonyl cyanide *m*-chlorophenylhydrazone or (○) with 150 μM-carbonyl cyanide *m*-chlorophenylhydrazone. ■, Uptake into nucleic acids. Carbonyl cyanide *m*-chlorophenylhydrazone was added 1 min before the [^{14}C]adenine. In drawing the curves a zero-time 'blank' of 18 pmol was assumed, and the time values plotted are 20s after the time of pouring on to the filters, since 20s was needed to filter the cells from the excess of incubation medium. No ethanol wash was used in this experiment.

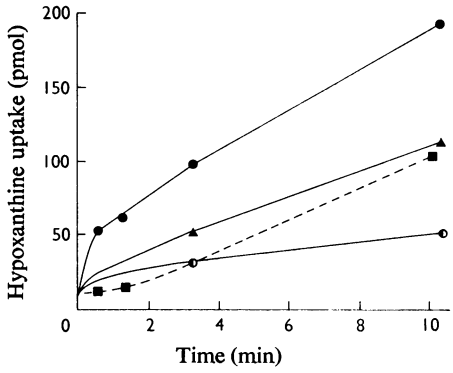

Fig. 8. *Uptake of hypoxanthine by strain SØ609 (hpt,gpt,pup)*

Conditions were as for Fig. 7, but [8-^{14}C]hypoxanthine (0.87 μM, 52 Ci/mol) was used and 1.53×10^9 cells. ▲, Uptake with washing; ●, uptake with no washing; ◐, 45 μM-carbonyl cyanide *m*-chlorophenylhydrazone 2 min before [^{14}C]hypoxanthine and no washing. ■, Uptake into nucleic acids.

spontaneous revertants of strain SØ446, which have recovered adenine phosphoribosyltransferase activity.

The analogous uptake of [8-^{14}C]hypoxanthine by strain SØ609 (*hpt,gpt,pup*) was also inhibited by carbonyl cyanide *m*-chlorophenylhydrazone, but most of the acid-soluble ^{14}C was lost from the bacteria by washing on the filters (Fig. 8). The residual acid-soluble ^{14}C appeared to be present both as hypoxanthine and as nucleotides, but reliable measurements were not obtained.

Strain SØ609 has adenine phosphoribosyltransferase and can use adenine readily. Likewise strain SØ446 will use hypoxanthine normally.

Concentration gradient of free adenine

The intracellular concentrations of free [^{14}C]-adenine detected in strain SØ446 represent an accumulation of 30-fold or less over the external concentration, assuming 2.7 μl of cell water/mg dry wt. of bacteria (Winkler & Wilson, 1966). This contrasts with a 900-fold accumulation of glycine seen in Fig. 4(*d*), or similar accumulations of cytidine and thymidine found by Munch-Petersen & Mygind (1976). However, the distribution of free [^{14}C]-adenine in strain SØ446 might underestimate the actual concentration gradient, because there can be an endogenous production of free adenine during the growth of similar strains (Kocharian *et al.*, 1975).

Roy-Burman & Visser (1975) reported a 144-fold concentration of free adenine in *E. coli* B, but this could possibly be an overestimate, owing to enzymic degradation of adenine nucleotides during the washing and extraction, or an underestimate, owing to metabolism of free adenine during washing. Since the major route for producing free adenine must be purine nucleoside phosphorylase, it is desirable to use other extraction methods and to study a *pup* strain. When the fate of [^{14}C]adenine was examined in strain SØ609, up to 9% of the ^{14}C extracted by 4 M-formic acid was found as free adenine, representing an internal/external concentration ratio of 90 at 10–15 s.

Concentration gradient of free hypoxanthine

Although the concentrations of hypoxanthine accumulated in strain SØ609 could not be measured, the internal concentration could not have been greater than 25 times the external concentration even if all the material that leaked out on washing had been free hypoxanthine.

Uptake of uracil in a strain without uracil phosphoribosyltransferase

Mutants in the *upp* gene lack uracil phosphoribosyltransferase and have been reported to take up negligible amounts of uracil (Beck & Ingraham, 1971; Pierard *et al.*, 1972). Uracil is, however, readily taken up by *E. coli* B, which has also been reported to lack the same enzyme (von Dippe *et al.*, 1975), but I can detect considerable activity in extracts of this strain in the presence of 10 mM-GTP. Molloy & Finch (1969) originally showed that GTP is a strong activator of uracil phosphoribosyltransferase in *E. coli*.

To investigate the possible role of this enzyme in uracil transport a *upp* strain, 4K3, known to be resistant to uracil analogues (Beacham & Pritchard, 1971), has been examined further. No activity of the uracil phosphoribosyltransferase was detected in the presence of 10 mM-GTP, and little [^{14}C]uracil was taken up even when measured without washing the bacteria. However, addition of inosine or adenosine facilitated rapid uptake of uracil, whereas deoxyinosine had virtually no effect (Fig. 9). Since the ^{14}C could be incorporated into nucleic acids if ammonium salts were supplied, the uracil was presumably metabolized by uridine phosphorylase (EC 2.4.2.3) and uridine kinase (EC 2.7.1.48), ribose 1-phosphate being generated by purine nucleoside phosphorylase, for which inosine is a better substrate than adenosine (Robertson & Hoffee, 1973). These results indicate that the uracil-transport system requires removal of intracellular uracil by either the phosphoribosyltransferase or uridine phosphorylase.

Cross-inhibition effects

In the *apt,pup* strain SØ446, 2.2 mM-adenine inhibited the uptake of 0.8 μM-hypoxanthine by 53%,

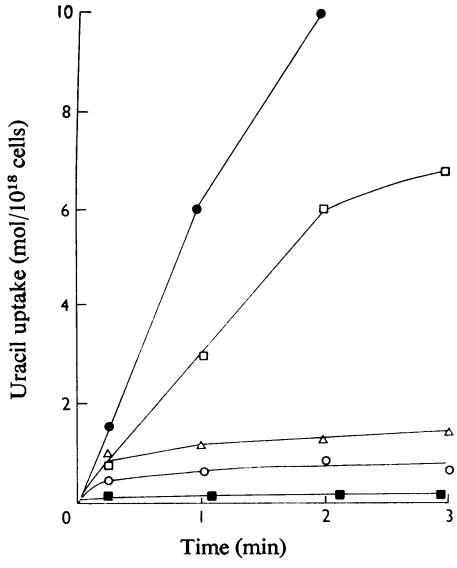

Fig. 9. *Effects of purine nucleosides on uracil uptake in a upp strain*

Conditions were as for Fig. 1, but 0.25 μM-[5-^{3}H]-uracil (89 μCi/litre) and 1.3×10^8 cells/ml were used. Other additions at zero time were: ○, none; ●, 4mM-inosine; □, 4mM-adenosine; △, 4mM-deoxyinosine; ■, 4mM-adenosine plus 30 μM-carbonyl cyanide *m*-chlorophenylhydrazone.

but 4mM-uracil had no effect. Likewise 0.73mM-hypoxanthine inhibited the uptake of 0.8 μM-adenine by 61% in strain SØ609 (*hpt,gpt,pup*), 4mM-uracil again having no effect. Because of the strains used, these inhibitions could not be due to competition for ribose phosphates or to feedback inhibition by nucleotides formed from the inhibitory purine. Uracil is apparently transported by a different system from those involved in hypoxanthine or adenine uptake.

The effect of hypoxanthine on adenine uptake is unlikely to be due to an action on adenine phosphoribosyltransferase, because 3mM-hypoxanthine inhibited this reaction by only about 15% when the concentration of adenine was only 10.18 μM. Thus hypoxanthine appears to inhibit the adenine-transport system directly. A reciprocal situation arises in the action of adenine on hypoxanthine uptake by strain SØ446. It is not yet possible to say if the two purines are transported by the same or by different porter systems.

Discussion

The findings in the present paper can be explained if adenine, hypoxanthine and uracil are taken up by transporter systems that are coupled to a protonmotive force, but are facilitated if the intracellular base is removed by a phosphoribosyltransferase. In the absence of such an enzyme, its function can be partly replaced by an appropriate nucleoside phosphorylase acting in the direction of nucleoside synthesis. Studies of spheroplasts have shown that the nucleoside phosphorylases are located intracellularly and not in the periplasmic space (Taketo & Kuno, 1972), despite the partial release of those enzymes by osmotic shock. This view has been elegantly confirmed by Munch-Petersen & Mygind (1976), who found that mutants that cannot transport nucleosides into the cell are unable to phosphorolyse nucleosides.

Although the factors that limit the intracellular accumulation of free bases are as yet poorly understood, appreciable concentration gradients of adenine are built up. There is therefore much evidence pointing to transport systems for the free bases and none to implicate a group-translocation process directly. On the contrary, the latter mechanism would not explain why the *uncA* strain, A103c, is more sensitive to uncouplers than is the nearly isogenic *uncA*$^+$ strain. At the concentrations used, the various uncouplers do not decrease the rate of O_2 consumption in strain A103c metabolizing glucose. Consequently they are not expected to inhibit the uptake of nucleic acid bases by any indirect effects on the concentrations of ATP or glycolytic intermediates. Glycine uptake occurs against a much greater concentration gradient of the transported solute, so that on thermodynamic grounds a higher protonmotive force is likely to be needed with perhaps a greater sensitivity to uncouplers. Yet there are general similarities in the sensitivities of the adenine, hypoxanthine, uracil or glycine uptake processes to uncouplers, though precise comparisons are difficult, since the rates of uptake, especially those for adenine in the presence of carbonyl cyanide *m*-chlorophenylhydrazone, can fall markedly during the first 2min of uptake.

Inhibition of certain transport processes by arsenate in an *uncA* strain has been taken to indicate a dependence on ATP (Berger & Heppel, 1974). The nature of this dependence is obscure and may even be regulatory (Lieberman & Hong, 1976). Arsenate could cause a deficiency of ribose phosphates either by arsenolysis of nucleosides or by inhibiting the synthesis of energy-rich phosphates. This would inhibit the removal of intracellular bases by nucleoside or nucleotide formation. It is not necessary to invoke a direct need for ATP in the translocation process.

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