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MECHANISM OF ENERGY COUPLING FOR TRANSPORT OF DEOXYCYTIDINE, URIDINE, URACIL, ADENINE AND HYPOXANTHINE IN *ESCHERICHIA COLI*

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Summary

The transport processes for uridine, deoxycytidine, uracil, adenine and hypoxanthine require an energy source and are active under anaerobic or aerobic conditions. Inhibitory effects of cyanide, arsenate, carbonylcyanide *m*-chlorophenylhydrazone, 2,4-dinitrophenol and *N,N'*-dicyclohexylcarbodiimide on the transport of uridine and deoxycytidine differ from the corresponding effects on the transport of uracil, adenine and hypoxanthine. The nature of these inhibitory effects supports the conclusion that uridine and deoxycytidine transport is energized either by electron transport or by ATP hydrolysis via (Ca^{2+} + Mg^{2+})-ATPase. The transport of uracil, adenine and hypoxanthine is dependent upon ATP or some high energy phosphate derivative of ATP, but is independent of (Ca^{2+} + Mg^{2+})-ATPase and electron transport. Uptake of the ribose moiety of uridine by a mutant of *Escherichia coli* B, which lacks the transport system for uracil and intact uridine, is neither stimulated by energy sources nor inhibited by various inhibitors of energy metabolism under either aerobic or anaerobic conditions.

Introduction

It is well known that several amino acids and carbohydrates are transported in *Escherichia coli* against a concentration gradient in unmodified form [1,2]. More recently, investigations from this laboratory [3—5] and others [6,7] have shown that certain nucleosides, pyrimidines and purines are accumulated intracellularly in unchanged form indicating an active transport process for these substrates. Little is known about how metabolic energy is coupled to the active

Abbreviations: CCCP, Carbonylcyanide *m*-chlorophenylhydrazone; DCCD, *N,N'*-dicyclohexylcarbodiimide.

transport of these bases and nucleosides relative to published information concerning the mechanism of energy coupling for the active transport of amino acids and carbohydrates in *E. coli*. Kaback [8] and Klein and Boyer [9] have shown that ATP and other high-energy phosphate compounds are not directly involved in active transport. Kaback and co-workers proposed [8,10] that active transport is directly coupled to a portion of the electron transport chain. More recently evidence has been presented indicating that anaerobic transport of β-galactosides and amino acids in *E. coli* whole cells and membrane vesicles is also driven by electron transfer [11,12]. Others have suggested that a high energy membrane state derived from either respiration or ATP hydrolysis via $(Ca^{2+} + Mg^{2+})$-ATPase is the immediate energy source for active transport in bacteria [9,13—15]. Other transport systems such as those for certain amino acids [14,15], D-ribose [16], glycylglycine [17] and galactose [18] transport systems utilize ATP or some high-energy compound derived from ATP to drive active transport. The latter transport systems do not utilize ATP hydrolysis via $(Ca^{2+} + Mg^{2+})$-ATPase.

It has recently been shown that strains of *E. coli* which cannot metabolize cytidine and uridine retain the capacity to effectively transport and concentrate these nucleosides several hundred-fold [19]. These studies firmly establish that nucleosides are transported, as such, by active transport systems and provide clear confirmation of earlier evidence for such transport systems [20—25].

The mechanism by which the free bases are transported is less clearly established. Hochstadt [26] has reviewed evidence that phosphoribosyltransferase enzymes are involved in a group translocation process in *E. coli* whereby the bases are transported into the cells as the nucleoside monophosphates. Others [4,27,28] presented evidence that the bases may be transported into the cells without participation of phosphoribosyltransferase enzymes. Jackman and Hochstadt [29] reported recently that there are two transport systems for hypoxanthine in *Salmonella typhimurium*; one transports the free base, the other involves phosphoribosyltransferase in a group translocation process. Although the precise mechanism involved in the transport of the bases is controversial, the assay conditions used in the present studies measure a single transport system in each case [4,5]. Therefore, the conclusions are applicable to either of the proposed mechanisms for base transport.

The objective of the present work was to investigate the means by which energy coupling occurs in the active transport of deoxycytidine, uridine, uracil, adenine and hypoxanthine in *E. coli* B. Various metabolic inhibitors were used to determine whether phosphate bond energy, electron transport, or an energized membrane state derived either from electron transport or ATP hydrolysis via membrane-bound $(Ca^{2+} + Mg^{2+})$-ATPase is the primary energy donor for their uptake.

Materials and Methods

Materials. All ^{14}C-labeled compounds were obtained from New England Nuclear. Tetrahydrouridine was supplied by Dr. Harry B. Wood, Drug Research and Development, Division of Cancer Treatment, National Cancer Institute.

Isolation of mutants and growth of cells. Two mutants of *E. coli* B, mutant

U^- and mutant U^-UR^-, were utilized to facilitate these studies. The transport characteristics and isolation of these mutants have been described by Leung and Visser [5]. Mutant U^- lacks the capacity to transport uracil whereas transport of nucleosides, purines and other bases is the same as in *E. coli* B. Mutant U^-UR^- lacks the uracil transport system as well as the ability to transport intact uridine. Both of these mutants retain the ability to transport the ribose moiety of uridine in an amount equal to that of the uracil moiety of uridine formed in the medium [5]. The transport of the ribose moiety by *E. coli* B is clearly a process which is distinct from the transport of ribose [5]. The parent and the mutant strains were grown aerobically at 37°C in minimal medium [30] with glucose as energy source. Acetate was used as a carbon source during growth of the cells when acetate was used in the uptake assays. Cells were harvested in midlog phase, washed twice by centrifugation in either medium A (minimal medium [30] without glucose) or medium B (0.1 mM Tris, 0.25 mM $MgSO_4$, 15 mM KCl, 7.5 mM $(NH_4)_2SO_4$, pH 7.0). Cells were resuspended in the same medium to a density of $5 \cdot 10^9$ cells/ml.

Assay for uptake. Unless otherwise noted, the standard reaction mixture (1 ml) contained parent strain *E. coli* B, mutant U^- or mutant U^-UR^- cell suspension (equivalent to 0.4—0.5 mg dry weight) in medium A or B. *E. coli* B was used when uptake of uracil, adenine, hypoxanthine or deoxycytidine was measured [3,4]. Mutant U^- was used when uptake of intact uridine was measured [5]. This mutant lacks the transport system for uracil, therefore measurement of uridine uptake is not complicated by the transport of uracil formed in the medium from uridine by periplasmic uridine phosphorylase [5]. The transport of the ribose moiety of uridine was measured by the use of mutant U^-UR^- [5], which lacks the transport system for uracil as well as intact uridine. Uptake of the ribose moiety of uridine by this mutant can be determined without concomitant uracil and intact uridine uptake. The assay mixtures were preincubated for 10 min at 37°C with or without the indicated energy source. The reaction was initiated by the addition of the ^{14}C-labeled base, uridine or deoxycytidine. Uptake of bases and uridine was measured after 30 s incubation at 37°C. Unless otherwise noted the uptake of deoxycytidine was measured after 1 min incubation at 37°C. In all experiments the substrate concentration for adenine, hypoxanthine and uracil was 5 μM and that for uridine and deoxycytidine was 20 μM. The specific activities of the radioactive substrates were as follows: [2-^{14}C]uracil, 2—50 Ci/mol; [8-^{14}C]adenine, 10—50 Ci/mol; [8-^{14}C]-hypoxanthine, 6—55 Ci/mol; [2-^{14}C]uridine, 2—50 Ci/mol; and deoxy[2-^{14}C]-cytidine, 1—29 Ci/mol. Inhibitors were added just prior to the preincubation period except for DCCD which was added to the cell suspension and incubated overnight at 4°C [9]. Tetrahydrouridine (205 μM), an inhibitor of periplasmic deoxycytidine deaminase [3], was added just prior to the preincubation period only when deoxycytidine was used as substrate. Aerobic uptake was measured in open test tubes with shaking. Anaerobic uptake was measured in closed test tubes containing a small port to allow for the escape of gases and the addition of substrate. Nitrogen was bubbled through the reaction mixture during the preincubation and incubation time periods. After incubation for the indicated period of time, the reactions were terminated and the cells were filtered, washed and counted, as described previously [22] except that the dilution of

the reaction mixtures and the washing procedures were carried out at room temperature.

The uracil found in the medium after incubation with [^{14}C]uridine has been reported to be equivalent to the amount of ribose moiety uptake in mutant U$^-$UR$^-$ [5]. Therefore, uptake of the ribose moiety of uridine in this mutant was measured as the amount of uracil excreted in the medium from [2-^{14}C]-uridine.

Analysis of products in E. coli cells and in medium after incubation with ^{14}C-labeled bases or nucleosides. Analysis of the intracellular and extracellular radioactive products was carried out as described previously [3,4,23] except that the medium was collected immediately after the indicated incubation time periods and heated in a boiling water bath for 2 min without prior dilution of the reaction mixture with medium A or B. Intracellular concentrations were based on an intracellular volume of 1 μl/0.4 mg dry weight [31].

Results

Uptake of deoxycytidine, uridine, uracil, adenine and hypoxanthine in the absence of energy sources under aerobic or anaerobic conditions is very low (Table I). Addition of acetate, lactate or glucose greatly increases the rate of

TABLE I

EFFECT OF ENERGY SOURCES ON THE UPTAKE OF DEOXYCYTIDINE, URIDINE, URACIL, ADENINE AND HYPOXANTHINE BY *E. COLI* B AND MUTANT U$^-$

Uptake was measured under standard assay conditions in medium A, with the indicated carbon sources. Acetate was used as the carbon source during growth of the cells when acetate was used in the uptake assays.

Strain	Substrate	Addition	Uptake (pmol/30 s)	
			+O_2	−O_2
E. coli B	Deoxycytidine	None	30	39
		5 mM glucose	1070	986 (1159) *
		15 mM acetate	1055 *	n.d.
		10 mM D-lactate	290	n.d.
Mutant U$^-$	Uridine	None	12	10
		5 mM glucose	1250	1110
		10 mM D-lactate	303	n.d.
E. coli B	Uracil	None	4	6
		5 mM glucose	352	104
		15 mM acetate	69	n.d.
		10 mM D-lactate	89	n.d.
E. coli B	Adenine	None	48	55
		5 mM glucose	787	771
		15 mM acetate	388	n.d.
		10 mM D-lactate	254	n.d.
E. coli B	Hypoxanthine	None	7	6
		5 mM glucose	724	432
		15 mM acetate	126	n.d.
		10 mM D-lactate	128	n.d.

* pmol/min.
n.d., not determined.

TABLE II

EFFECT OF KCN ON THE UPTAKE OF DEOXYCYTIDINE, URIDINE, URACIL, ADENINE AND HYPOXANTHINE BY *E. COLI* B AND MUTANT U⁻

Uptake was measured under standard assay conditions in medium A and in the presence of the indicated concentrations of KCN. Cells used for the assays were grown in the presence of glycerol. 10 mM lactate was used for assays under aerobic conditions and 5 mM glucose was used for assays under anaerobic conditions.

Strain	Substrate	KCN (mM)	Uptake (%)	
			$+O_2$	$-O_2$
E. coli B	Deoxycytidine	None	100 (290) *	100 (718) *
		0.2	23	95
		10	20	90
		20	10	n.d.
Mutant U⁻	Uridine	None	100 (303) *	100 (1298) *
		0.2	13	86
		10	6	90
E. coli B	Uracil	None	100 (89) *	100 (110) *
		0.2	10	93
		10	7	76
		20	n.d.	75
E. coli B	Adenine	None	100 (403) *	100 (798) *
		0.2	7	99
		10	2	100
		20	n.d.	80
E. coli B	Hypoxanthine	None	100 (125) *	100 (432) *
		0.2	6	n.d.
		10	1	100
		20	n.d.	75

* pmol/30 s.
n.d., not determined.

uptake, although the stimulatory effect is somewhat variable and dependent upon the energy source.

It has been reported that oxidative phosphorylation is depressed in *E. coli* B when cells are grown in the presence of glucose [32], but not in cells grown in acetate or glycerol. Therefore, the effects of acetate, lactate and glucose on uptake of each of the substrates were compared in cells grown in glycerol, acetate and glucose. It was determined that the energy source utilized for growth of the cells does not influence the transport properties of these cells.

Aerobic uptake of deoxycytidine, uridine, uracil, adenine and hypoxanthine is inhibited about 80—90% by 0.2 mM KCN (Table II). Anaerobic uptake of the bases and nucleosides is only slightly inhibited in the presence of 0.2 mM KCN, and even in the presence of 20 mM cyanide uptake is inhibited only about 20%.

The anaerobic and aerobic uptake of adenine, hypoxanthine and uracil is strongly inhibited by arsenate (Table III). However, the inhibitory effect of arsenate on deoxycytidine and uridine uptake is much more pronounced under anaerobic conditions than under aerobic conditions. The transport of the bases and the nucleosides is stimulated by the presence of phosphate ion at anaerobic and aerobic conditions (Table III). Deoxycytidine uptake is more markedly

TABLE III

EFFECT OF ARSENATE AND PHOSPHATE ON THE UPTAKE OF DEOXYCYTIDINE, URIDINE, URACIL, ADENINE AND HYPOXANTHINE BY *E. COLI* B AND MUTANT U⁻

The uptake was measured under standard assay conditions in medium B and in the presence of the indicated concentrations of potassium phosphate or potassium arsenate. Aerobic uptake was measured in the presence of 15 mM acetate and anaerobic uptake was measured in the presence of 5 mM glucose.

Substrate	Phosphate (mM)	Arsenate (mM)	Uptake (%)	
			$+O_2$	$-O_2$
Deoxycytidine	66	0	100	100
	0	0	34	8
	0	1	9	6
	0	10	3	1
	13.2	0	87	62
	13.2	10	64	18
Uridine	66	0	100	100
	0	10	11	5
	6.6	0	84	66
	6.6	10	38	19
	13.2	0	85	100
	13.2	10	54	24
Uracil	66	0	100	100
	0	0	49	77
	0	1	7	7
	0	10	0	0
Adenine	66	0	100	100
	0	0	37	73
	0	1	10	5
	0	10	10	4
Hypoxanthine	66	0	100	100
	0	0	45	64
	0	0.5	5	8
	0	1	2	4

TABLE IV

EFFECT OF ARSENATE ON INTRACELLULAR DISTRIBUTION OF RADIOACTIVITY DERIVED FROM DEOXY[^{14}C]CYTIDINE

Uptake was measured under standard assay conditions in medium B and in the presence of 13.2 mM phosphate and the indicated concentrations of arsenate. Incubation was for 1 min. The energy sources for uptake studies were 15 mM acetate for the aerobic assays and 5 mM glucose for the anaerobic assays. Distribution of intracellular radioactivity was determined as indicated in the text.

Arsenate	O_2	Total uptake (pmol)	Nucleotides (μM) *	Deoxycytidine (μM) *	Uracil (μM) *
0	+	983	564	217	201
10	+	723	188	265	270
0	—	907	538	203	228
10	—	194	43	50	101

* Intracellular concentrations are based on an internal volume of 1 μl/0.4 mg dry weight of the cells [31].

TABLE V

EFFECT OF ARSENATE ON INTRACELLULAR DISTRIBUTION OF RADIOACTIVITY DERIVED FROM [^{14}C]ADENINE

Uptake was measured under standard assay conditions in medium B and in the presence of the indicated amounts of arsenate and phosphate. The energy sources were 15 mM acetate for uptake under aerobic conditions and 5 mM glucose for uptake under anaerobic conditions. The distribution of intracellular radioactive compounds was determined as indicated in the text.

Arsenate (mM)	Phosphate (mM)	O_2	Total uptake (pmol)	Adenine (μM) *	Adenosine (μM) *	Hypo-xanthine (μM) *	Inosine (μM) *	Nucleo-tide (μM) *
0	13.2	+	250	20	6	1	3	220
10	13.2	+	82	5	2	1	1	73
0.5	0	+	28	3	1	3	1	19
0	13.2	—	951	40	30	34	22	815
10	13.2	—	371	6	9	4	9	342
0.5	0	—	22	4	1	1	2	14

* Intracellular concentrations are based on a internal volume of 1 μl/0.4 mg dry weight of the cells [30].

TABLE VI

EFFECT OF DCCD ON THE UPTAKE OF URIDINE, DEOXYCYTIDINE, URACIL, ADENINE AND HYPOXANTHINE BY *E. COLI* B AND MUTANT U$^-$

Uptake was measured under standard assay conditions in medium A and in the presence of 5 mM glucose. Cell suspensions were incubated overnight at 4°C with an ethanolic solution of DCCD at the indicated concentrations prior to the addition of glucose according to the procedure of Klein and Boyer [9]. The final ethanol concentration was 0.1% in all samples. The incubation time was 30 s.

Strain	DCCD (mM)	Substrate	Uptake (%)	
			+O_2	—O_2
Mutant U$^-$	None	[2-^{14}C]uridine	100	100
	0.1		87	53
	0.5		74	24
Mutant U$^-$	None	Deoxy[2-^{14}C]cytidine	100	100
	0.1		104	46
	0.5		86	18
E. coli B	None	Deoxy[2-^{14}C]cytidine	100 *	100 *
	0.01		90 *	17 *
	0.1		87 *	6 *
E. coli B	None	[2-^{14}C]uracil	100 (100) *	100
	0.1		45 (23) *	95
	0.5		19 (7) *	66
E. coli B	None	[8-^{14}C]adenine	100 (100) *	100
	0.1		72 (26) *	85
	0.5		57 (6) *	51
E. coli B	None	[8-^{14}C]hypoxanthine	100	100
	0.1		75	50
	0.5		45	50

* 15 mM acetate was used in the incubation mixture in place of 5 mM glucose for aerobic uptake. The incubation time for deoxycytidine was 1 min and the cells were preincubated with DCCD at 25°C for 3 h before addition of the energy sources.

reduced by low levels of phosphate than uptake of bases or uridine.

Arsenate causes a marked decrease of uptake into intracellular uracil, deoxycytidine and nucleotides after incubation of cells with deoxy[2-^{14}C]cytidine under anaerobic conditions (Table IV). It also results in an almost complete elimination of the deoxycytidine concentration gradient. Under aerobic conditions radioactivity found as intracellular nucleotides is reduced to about one-third by arsenate, but radioactivity in intracellular uracil and deoxycytidine fractions is somewhat increased by arsenate.

Radioactivity found in intracellular adenine, adenosine, hypoxanthine, inosine and nucleotides after incubation with [^{14}C]adenine is drastically reduced by arsenate under both anaerobic and aerobic conditions (Table V). The concentration gradient of adenine is completely eliminated by arsenate under both conditions.

The effect of DCCD on the uptake of deoxycytidine, uridine, uracil, adenine and hypoxanthine was studied to investigate a possible role of (Ca^{2+} + Mg^{2+})-ATPase in the active transport processes. DCCD markedly reduces anaerobic uptake of deoxycytidine and uridine and has relatively little effect on their aerobic uptake (Table VI). The concentrations of DCCD which are effective for inhibition of anaerobic transport of the nucleosides are in the same range as those required for inhibition of *E. coli* B (Ca^{2+} + Mg^{2+})-ATPase [33]. The inhibitory effect of DCCD on the anaerobic uptake of uracil, adenine and hypoxanthine (Table VI) is very low as compared to its inhibitory effects on uridine

TABLE VII

EFFECT OF ENERGY POISONS ON THE UPTAKE OF DEOXYCYTIDINE, URIDINE, URACIL, ADENINE AND HYPOXANTHINE BY *E. COLI* B AND MUTANT U$^-$

Uptake was measured under standard assay conditions in medium A and in the presence of 15 mM potassium acetate (aerobic uptake) or 5 mM glucose (anaerobic uptake). [2-^{14}C]Uridine uptake by mutant U$^-$, at aerobic and anaerobic conditions, was measured in the presence of 5 mM glucose. DNP, dinitrophenol.

Strain	Substrate	Inhibitor	Uptake (%)	
			$+O_2$	$-O_2$
E coli B	Deoxycytidine	None	100 (1055) *	100 (1159) *
		2 mM DNP	14	20
		15 μM CCCP	1	3
Mutant U$^-$	Uridine	None	100 (1250) **	100 (1010) **
		2 mM DNP	17	2
		15 μM CCCP	5	3
E. coli B	Uracil	None	100 (50) **	100 (120) **
		2 mM DNP	4	11
		7.5 μM CCCP	22	50
		15 μM CCCP	7	14
E. coli B	Adenine	None	100 (331) **	100 (758) **
		2 mM DNP	15	21
		15 μM CCCP	12	43
E. coli B	Hypoxanthine	None	100 (126) **	100 (440) **
		2 mM DNP	21	6
		15 μM CCCP	3	4

* pmol/min.
** pmol/30 s.

TABLE VIII

EFFECT OF VARIOUS ENERGY POISONS ON UPTAKE OF THE RIBOSE MOIETY OF URIDINE BY MUTANT U⁻UR⁻

Uptake was measured under standard assay conditions in medium A and in the presence of 5 mM glucose and the indicated additions. Incubation was for 30 s. Uptake of the ribose moiety of [2-^{14}C]uridine was determined as the amount of uracil formed in the medium during the incubation period as described in the text. Conditions for treatment with DCCD are the same as those for Table II.

Addition	Concentration (mM)	Uptake (%)	
		$+O_2$	$-O_2$
None	—	100 (3560) *	100 (3300) *
KCN	1	88	100
KCN	2	84	113
KCN	10	n.d.	98
DCCD	0.1	106	100
DCCD	0.5	78	70
Dinitrophenol	1	82	70
CCCP	0.015	50	50
None (—phosphate)	—	97 **	109 **
Arsenate (—phosphate)	10	98 **	99 **
None (—glucose)	—	97	98

* pmol/30 s.

** Uptake was measured under standard assay conditions in medium B.

n.d., not determined.

and deoxycytidine uptake under anaerobic conditions.

Uncouplers of oxidative phosphatorylation such as 2,4-dinitrophenol and CCCP dissipate the energized membrane state. These inhibitors were used to determine whether the transport processes utilize this state directly as an energy source. The data (Table VII) show that uptake of deoxycytidine, uridine and free bases is inhibited more than 80% by 2,4-dinitrophenol and more than 90% by CCCP under aerobic or anaerobic conditions.

Uptake of the ribose moiety by mutant U⁻UR⁻ is similar under both aerobic and aearobic conditions and is not stimulated by glucose (Table VIII). In constrast to nucleoside and base transport, the uptake of the ribose moiety is not significantly inhibited by KCN, DCCD, 2,4-dinitrophenol or arsenate under aerobic or anaerobic conditions and the uptake is the same in the presence or absence of phosphate (Table VIII).

Discussion

The nature of the effects of the various inhibitors supports the conclusion that uridine and deoxycytidine transport is energized either by electron transport or by ATP hydrolysis via (Ca^{2+} + Mg^{2+})-ATPase. On the other hand, the transport of uracil, adenine and hypoxanthine is dependent upon ATP or some high energy phosphate derivative of ATP, but is independent of (Ca^{2+} + Mg^{2+})-ATPase. Electron transport does not energize transport of these free bases. The data which support these conclusions are given below.

The transport of uridine and deoxycytidine under aerobic conditions is almost completely inhibited by KCN, suggesting that either a high energy phos-

phate bond or electron transport, or both, can be utilized as an energy donor for their transport (Table II). Since uridine and deoxycytidine are transported efficiently under anaerobic conditions in the presence of glucose, it is clear that respiration is not an absolute requirement (Table I) and that ATP derived from glycolysis can act directly or indirectly as an energy source. This conclusion is also demonstrated by the inhibitory effect of arsenate (Tables III and IV) which is known to drastically reduce intracellular ATP levels [9]. Arsenate causes a marked decrease of all intracellular components under anaerobic contions and also results in an almost complete elimination of the deoxycytidine concentration gradient (Table IV). The strong inhibitory effect of DCCD on deoxycytidine and uridine transport under anaerobic conditions (Table VI) is evidence that ATP provides the high energy membrane state necessary for transport by hydrolysis of ATP via (Ca^{2+} + Mg^{2+})-ATPase.

Arsenate also inhibits aerobic uptake of these nucleosides but to a much lesser extent than under anaerobic conditions (Table III). This relatively low inhibitory effect of arsenate can be explained by a slow rate of nucleotide formation subsequent to nucleoside transport in the presence of the inhibitor. Deoxycytidine and uridine are rapidly converted to nucleotides after their transport into cells [3,5], but in the presence of arsenate, the low intracellular ATP concentration results in a reduction of total uptake (retention) due to a decrease in nucleoside phosphorylation (Table IV). Under aerobic conditions, however, the concentration gradient of deoxycytidine is maintained (Table IV), indicating that ATP is not an absolute requirement for energizing the active transport process and that electron transport may be utilized as an alternative energy source to provide a high energy membrane state necessary for active transport to occur. This conclusion is supported by the demonstration that the aerobic transport of these nucleosides is not inhibited appreciably by DCCD (Table VI).

Uncouplers of oxidative phosphorylation such as dinitrophenol and CCCP are presumed to act by either preventing the formation of an energized membrane state or causing its dissipation. Therefore, transport systems which are dependent upon the energized membrane state are inhibited by uncouplers whether the energy is derived from respiration or ATP hydrolysis via (Ca^{2+} + Mg^{2+})-ATPase [9]. The strong inhibitory effect of dinitrophenol and CCCP on the transport of deoxycytidine and uridine under both aerobic and anaerobic conditions (Table VII) provides additional evidence that the high energy membrane state used to drive the active transport of the nucleosides can be derived either from respiration or ATP hydrolysis by (Ca^{2+} + Mg^{2+})-ATPase. Similar systems for the active transport of certain amino acids [9,14,15] and carbohydrates [9] in *E. coli* have been reported previously.

A number of transport systems in *E. coli* have been described which do not involve the (Ca^{2+} + Mg^{2+})-ATPase, but are energized directly by ATP or high energy compounds derived from ATP. These include the shock-sensitive transport systems for certain amino acids [14,15], D-ribose [16], glycylglycine [17] and galactose [18]. The present results provide evidence for similar transport systems for uracil, adenine and hypoxanthine. The strong inhibitory effects of arsenate on the uptake of uracil, adenine and hypoxanthine under aerobic as well as anaerobic conditions (Table III) indicate a requirement for ATP

under both aerobic and anaerobic conditions. Arsenate not only drastically reduces the concentration of all intracellular components derived from adenine but also completely eliminates the concentration gradient of adenine under both aerobic and anaerobic conditions (Table V). Therefore, the inhibitory effect of cyanide (Table II) on uptake of uracil, adenine and hypoxanthine may be explained by the reduced concentration levels of ATP formed in the presence of this inhibitor rather than by inhibition of electron transport.

The ATP requirement for transport of the free bases does not involve (Ca^{2+} + Mg^{2+})-ATPase since the anaerobic uptake of free bases is not significantly inhibited by DCCD (Table VI). A requirement for ATP production under aerobic conditions is also indicated by the strong inhibition of transport by the uncouplers, dinitrophenol and CCCP (Table VII). Anaerobic transport of the bases is also inhibited by uncouplers (Table VII). This inhibitory effect is much lower and may be explained by non-specific effects of the uncouplers which have been reported previously by others rather than by their ability to dissipate the energized membrane state. For example, the inhibitory effect of dinitrophenol and carbonylcyanide *p*-trifluoromethoxyphenylhydrazone on transport of glutamine [14,15], D-ribose [16] and glycylglycine [17] has been explained by the low ATP levels produced by these uncouplers even under conditions when ATP is generated solely by glycolysis [15]. The transport processes for these compounds, like the free bases, were shown to require ATP or some high-energy derivative of ATP without involvement of (Ca^{2+} + Mg^{2+})-ATPase.

In contrast to the data obtained on the uptake of intact uridine, the transport of the ribose moiety of uridine by mutant U^-UR^- is not significantly stimulated by energy sources and is not inhibited by any of the inhibitors (Table VIII). These results show that neither respiration nor phosphate bond energy is required for its transport. It may be concluded that the transport system for the ribose moiety of uridine differs markedly from that of intact uridine, and also differs from that of D-ribose which requires ATP as an immediate energy source [16].

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