Deoxycytidine Transport in the Presence of a Cytidine Deaminase Inhibitor and the Transport of Uracil in *Escherichia coli* B*

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**SUMMARY**

Tetrahydrouridine, a cytidine deaminase inhibitor, prevents periplasmic degradation of deoxycytidine by *Escherichia coli* B. It does not inhibit deoxycytidine transport and therefore allows an accurate determination of deoxycytidine transport. Data obtained using tetrahydrouridine show that deoxycytidine is transported in *E. coli* B as the intact nucleoside by an active transport process, with a \( K_m \) of \( 5 \times 10^{-7} \) M. Cytidine and deoxyadenosine inhibit transport competitively, whereas guanosine has no effect on transport. Arsenate or KCN greatly reduces transport. In a mutant resistant to the deaminase, it can be demonstrated that uracil appears rapidly in the medium when cells are incubated for short time periods in the presence of cytidine (3).

Rapid conversion of nucleosides to deaminated products and free bases by *Escherichia coli* leads to difficulties in accurate quantitative estimation of nucleoside transport in whole cells, particularly since these conversions may occur in the periplasmic space. Thus, determinations of uridine transport are complicated by a periplasmic degradation of uridine to uracil. This uracil contributes to an unknown extent to observed uptake from uridine (1). In addition, rapid degradation of substrate in the medium prevents measurement of accurate kinetic parameters for transport.

Similar problems are associated with quantitation of cytidine and deoxycytidine transport. These nucleosides are not subject to phosphorolysis but are rapidly deaminated and then degraded to the free base. Cytidine deaminase is found in cell-free extracts (2) and is generally considered to be a cytoplasmic enzyme (3). However, its presence in shock fluid from *E. coli* has been reported by Hochstadt (4). Irrespective of the location of the deaminase, it can be demonstrated that uracil appears rapidly in the medium when cells are incubated for short time periods in the presence of cytidine (3).

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**Materials and Methods**

**Materials**—The \(^{14}C\)-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 Showdomycin-resistant Mutants and Growth of Cells—** An *Escherichia coli* B (Hill) mutant, resistant to 80 \( \mu \)M showdomycin, was developed as described previously (6). Two mutant and parent cells used in all experiments were grown in minimal medium (7) in the absence of showdomycin and were harvested as described previously (6).

**Assay for Uptake—** Unless otherwise noted, the standard reaction mixture (1 ml) contained a cell suspension of *E. coli* B or the showdomycin-resistant mutant (equivalent to 0.4 to 0.5 mg dry weight) in Medium A (Davis and Mingioli medium without glucose) (7). The cells were preincubated without substrate for 15 min at 37°C. When glucose or tetrahydrouridine was added, the addition was made immediately prior to the preincubation period. The reaction was initiated by addition of \(^{14}C\)-labeled substrate, and incubation continued at 37°C for the indicated time period. Specific activity of \([2-^{14}C]\)deoxycytidine varied from 1.4 Ci/mmol to 29.7 Ci/mmol. Specific activity of \([2-^{14}C]\)uracil ranged from 10 Ci/mol to 50 Ci/mol. Heterologous nucleosides, when present, were added together with the substrate. Cells were collected and washed on Millipore filters as described previously (6). Measurements of radioactivity were carried out as described previously (1). Uptake at zero time of incubation was subtracted from the results.

**Analysis of Products of Deoxycytidine Uptake in *E. coli* B and of Degradation Products in the Medium—** Analysis of the medium was performed as described previously for uridine (1), with separation of degradation products by descending chromatography in 86% 1-butanol in water (v/v). Where uridine, uracil, and deoxycytidine 

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1 The abbreviations used are: H, TID, 3,4,5,6-tetrahydrouridine; dCyD, 2'-deoxycytidine.
were separated, two-dimensional chromatography on Whatman No. 3MM paper was performed in 80% 1-butanol followed by 62.5% isobutyric acid and 37.5% 0.5 N NH₄OH (v/v), pH 3.6. Intracellular products were analyzed as described previously (1), except that separation was effected by chromatography in 80% 1-butanol and in 62.5% isobutyric acid-ammonia as above, and distribution of radioactivity at zero time of incubation was subtracted from the values obtained after 10 s or 1 min of incubation. Values obtained in the two solvent systems were averaged and are in good agreement. Intracellular concentrations were based on an intracellular volume of 1 μl/0.4 mg dry weight (8).

**Assay for Adenine and Uracil Phosphoribosyltransferase—**Cells grown as described above were sonified for 3 min. Activity was assayed in the crude extract, and in the supernatant from 10% streptomycin sulfate precipitation.

Adenine phosphoribosyltransferase was assayed by the method of Hochstadt-Ozer and Stadtman (9). Uracil phosphoribosyltransferase was assayed by the method of Crawford et al. (10), except that uracil and Mg₂⁺ α-5-phosphoribosyl-1-pyrophosphate concentrations were increased to 1 mM and 2 mM, respectively, and radioactive substrates and products were separated by thin layer chromatography on cellulose in 70% isopropyl alcohol, 10% NH₄OH, and 30% water (v/v).

**RESULTS**

**Effect of Tetrahydrouridine on Cytidine Uptake—**Tetrahydrouridine, added to the standard assay at concentrations ranging from 41 μM to 410 μM, has no effect on uptake of 250 μM [2-¹⁴C]cytidine during a 5-min incubation period. Similarly, 205 μM tetrahydrouridine has no effect on uptake of either 200 μM [2-¹⁴C] or [U-¹⁴C]uridine during a 1-min incubation period. Since uridine has been reported to inhibit cytidine uptake competitively (6), these preliminary data indicate that tetrahydrouridine does not compete for the transport of either of these nucleosides.

Uptake of 250 μM cytidine was found to be linear for 1 min. Fig. 1 shows uptake of various concentrations of cytidine during a 1-min incubation period in the presence of 250 μM tetrahydrouridine, and in the presence or absence of 2500 μM deoxycytidine. Cytidine transport consists of a high affinity saturable component and a component that shows no evidence of saturation at 1 mM cytidine concentration (Fig. 1), and a Lineweaver-Burk plot of its uptake is biphasic. The high affinity transport is completely inhibited by 2500 μM deoxycytidine, while the slower transport is not affected. The slower transport may therefore represent either a very low affinity transport system that is not inhibited by deoxycytidine or it may represent passive diffusion.

Deoxycytidine uptake (Fig. 1), on the other hand, gives a linear Lineweaver-Burk plot. It is possible that a second very low affinity system for deoxycytidine transport may exist as it does for cytidine. However, the much higher transport rate of deoxycytidine obviates the necessity of taking such a component into account, since it comprises such a minor portion of the total uptake at these concentrations. Therefore deoxycytidine transport was studied extensively in preference to cytidine transport.

**Effect of Tetrahydrouridine on Deoxycytidine Uptake—**The effect of varying concentrations of tetrahydrouridine on deoxycytidine uptake was determined (Table I). Tetrahydrouridine appears to inhibit uptake of 0.5 μM deoxycytidine in the presence of glucose, with maximum inhibition at 82 μM tetrahydrouridine. At higher deoxycytidine concentrations, or in the absence of glucose, tetrahydrouridine appears to stimulate deoxycytidine uptake. These results are verified by the data of Table I, which show the distribution of radioactivity from deoxycytidine, intracellularly and in the medium. It is evident (Table I) that a considerable fraction of deoxycytidine is degraded and appears as uracil in the medium, and tetrahydrouridine largely prevents this appearance of degradation products in the medium. At all concentrations of deoxycytidine, uracil represents the major fraction (93 to 100%) of the degradation products in the medium, the remainder consisting of uridine and deoxyuridine. Intracellular concentrations of uracil and uridine as well as total nucleotides are generally higher in the presence of tetrahydrouridine than in its absence. It is therefore apparent that intracellular breakdown of deoxycytidine is not prevented by 205 μM tetrahydrouridine. Although tetrahydrouridine prevents the rapid disappearance of deoxycytidine, it does not completely block the appearance of uracil in the medium. It is possible that the release of small molecules by the cold shock effect found by Leder (11) and other

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**TABLE I**

**Effect of tetrahydrouridine on deoxycytidine uptake by Escherichia coli B**

Uptake was measured by the standard assay. Cells corresponding to 0.4 mg dry weight were suspended in 1 ml of Medium A, with or without the addition of 5 mM glucose, together with the indicated concentrations of H₄Urd and [2-¹⁴C]deoxycytidine. Incubation was for 10 s at 37°C.

| dCyd concentration | 0.5 μM (+ glucose) | 20 μM (+ glucose) | 7.5 μM (-- glucose) |
|--------------------|--------------------|--------------------|--------------------|
| No HUrd            | 0.10               | 0.40               | 0.06               |
| 41 μM HUrd         | 0.09               | nd                 | 0.06               |
| 82 μM HUrd         | 0.06               | nd                 | 0.53               |
| 205 μM HUrd        | nd                 | nd                 | 0.08               |

*nd = not determined.*
TABLE II

Distribution of radioactivity from [2-14C]deoxycytidine inside cell and in medium

Uptake was measured by the standard assay after 10 s or 1 min of incubation with the indicated additions. Distribution of radioactivity was determined as described in the text.

| Initial [2-14C] dCyd | Additions | Total uptake | Inside the cell | In the medium |
|----------------------|-----------|--------------|----------------|--------------|
|                      |           |              | Nucleotides | dCyd | Uracil plus | dCyd | Uracil |
| µM                   |           |              | µmol | µM | µM | µM | µM |
| 0.5                  | 5 mM glucose, 205 µM H4Urd | 53 | 52 | 0 | 3 | 0.43 | 0.005 |
|                      | (10 s)   |              |              |      |    |      |      |
| 0.5                  | 5 mM glucose | 94 | 82 | 6 | 6 | 0.20 | 0.20 |
|                      | (10 s)   |              |              |      |    |      |      |
| 20                   | 5 mM glucose, 205 µM H4Urd | 540 | 317 | 101 | 120 | 19.0 | 0.19 |
|                      | (10 s)   |              |              |      |    |      |      |
| 20                   | 5 mM glucose | 420 | 260 | 80 | 80 | 12.0 | 7.1 |
|                      | (10 s)   |              |              |      |    |      |      |
| 7.5                  | 205 µM H4Urd | 83 | 5 | 49 | 29 | 7.4 | 0.1 |
|                      | (10 s)   |              |              |      |    |      |      |
| 7.5                  | none      | 62 | 5 | 35 | 24 | 4.6 | 2.8 |
|                      | (10 s)   |              |              |      |    |      |      |
| 200                  | 205 µM H4Urd | 710 | 61 | 479 | 170 | 200 | 1.8 |
|                      | (10 s)   |              |              |      |    |      |      |
| 200                  | none      | 530 | 45 | 365 | 124 | 120 | 83 |
|                      | (10 s)   |              |              |      |    |      |      |
| 7                    | 5 mM glucose, 205 µM H4Urd | 1750 | 1274 | 204 | 2724 | 5.1 | 0.34 |
|                      | (1 min)  |              |              |      |    |      |      |
| 7                    | 5 mM glucose | 1190 | 1046 | 90 | 55 | 0.1 | 6.7 |
|                      | (1 min)  |              |              |      |    |      |      |
| 30                   | 205 µM H4Urd | 750 | 48 | 192 | 506 | 29.2 | 0.50 |
|                      | (1 min)  |              |              |      |    |      |      |
| 30                   | none      | 330 | 30 | 165 | 131 | 8.2 | 22 |
|                      | (1 min)  |              |              |      |    |      |      |

* Including all phosphorylated nucleoside derivatives and nucleic acids.

* Figures in parentheses indicate time of incubation.

* Based on an intracellular volume of 1 µl/0.4 mg dry weight.

* Uracil concentration determined to be 50 µM, and uracil concentration 213 µM.

investigators (12) may account for a fraction of the uracil found in the medium. However, there is no relationship between the concentrations of uracil found in the medium and the uracil concentration in the cell. On the other hand, the low levels of uracil in the medium when tetrahydrouridine is present are in all cases directly related to the extracellular concentration of deoxycytidine and represent only about 1% of deoxycytidine (Table II). It may be concluded that a very low rate of deamination occurs at the membrane at the experimental conditions used, since very high intracellular concentrations of uracil can be maintained.

At the higher substrate concentrations used, the uptake of deoxycytidine is lower in the absence of tetrahydrouridine than in its presence (Table II). This result can be explained by the rapid disappearance of deoxycytidine in the medium due to periplasmic degradation when tetrahydrouridine is absent. Uptake is approximately proportional to the 5-s concentration of deoxycytidine obtained by integration of the first order rate equation for substrate disappearance in all cases, except at 0.5 µM deoxycytidine.

A deoxycytidine concentration gradient of 40:1 can be produced in the presence of glucose. Glucose stimulates markedly the cellular uptake both in the presence and absence of tetrahydrouridine (Table II).

Since tetrahydrouridine causes an inhibition of total uptake only at low (0.5 µM) deoxycytidine concentration, it was important to verify that this result is not related to an inhibition of uptake by the analog. Therefore, the uptake at 0.5 µM deoxycytidine was investigated in greater detail. By comparison of the uptake of [2-14C]- and [U-14C]deoxycytidine, the retention of the pyrimidine and deoxyribose moieties of deoxycytidine can be determined. It was found (Table III) that uptake of the deoxyribose moiety is lower in the absence of tetrahydrouridine than in its presence, whereas uptake of the pyrimidine moiety is greater.

Thus, the increased uptake of radioactive observed at 0.5 µM [14C]deoxycytidine in the absence of tetrahydrouridine is entirely accounted for by an increase in uptake of the pyrimidine moiety.

Uptake of the deoxyribose and pyrimidine moieties is equal in the presence of tetrahydrouridine, indicating that deoxycytidine is transported as the intact nucleoside and substantiating the conclusion that no loss of either pyrimidine or deoxyribose moieties occurs after transport into the cell. Furthermore, the increased uptake of the pyrimidine moiety at 0.5 µM deoxycytidine must represent transport of uracil, which is formed in the periplasmic space only in the absence of tetrahydrouridine. This contribution of uracil transport in the absence of tetrahydrouridine is significant only at low deoxycytidine concentrations and accounts entirely for the apparent inhibition of total uptake by tetrahydrouridine at 0.5 µM deoxycytidine as discussed below.

**Uracil Transport in E. coli B** - The transport of uracil in E. coli B was studied to elucidate further the effect of uracil on total uptake from [14C]deoxycytidine. Fig. 2 shows uptake of [2-14C]-uracil by E. coli B cells in the presence of glucose. Uptake follows Michaelis-Menten kinetics, giving a $K_m$ of $5 \times 10^{-7}$ M, and a $V_{max}$ of 0.18 nmol/8 s. In the absence of glucose, uracil uptake is...
FIG. 2. Uracil uptake by Escherichia coli B. Uptake was measured by the standard assay with the addition of 5 mM glucose. Incubation was for 8 s at 37°C.

TABLE IV

| Initial [3-14C]uracil concentration | Time of incubation | Total uptake | Inside the cell | Medium (uracil) |
|------------------------------------|--------------------|--------------|----------------|----------------|
|                                    | µM                 | µmol         | Uracil         | µM             |
| 0.16                               | 8 s                | 43           | 43.4           | 3.3            | 1.2            | 0.112          |
| 0.407                              | 8 s                | 101          | 87.3           | 10             | 4.9            | 0.306          |
| 1.63                               | 8 s                | 168          | 149            | 12             | 6.8            | 1.46           |
|                                    | 1 min              | 1310         | 1220           | 56             | 28             | 0.321          |
| 9.8                                | 8 s                | 175          | 146            | 12             | 18             | 9.62           |
| 9.8                                | 1 min              | 1550         | 1410           | 66             | 72             | 8.25           |

a including all phosphorylated nucleoside derivatives and nucleic acids.
b Based on an intracellular volume of 1 µl/0.4 mg dry weight.

less than 1% of that in the presence of glucose. Distribution of radioactivity from [3-14C]uracil (Table IV) shows that the major intracellular component is the nucleotide fraction at all time periods. Uracil can be concentrated intracellularly, and an inside to outside gradient of approximately 90:1 can be maintained after a 1-min incubation period.

These data on uracil uptake lead to the conclusion that the apparent inhibition of 0.5 µM deoxycytidine uptake by tetrahydrodridine can be explained by transport of extracellular uracil, which is present in the medium in the absence of tetrahydrodridine. Uracil is transported by an energy-dependent system with a high affinity and at a low maximum velocity (Fig. 2) compared with deoxycytidine transport (Fig. 3). Uracil and deoxycytidine concentrations at 5 s can be calculated from the data in Table II for an initial deoxycytidine concentration of 0.5 µM in the absence of tetrahydrodridine. These concentrations of uracil and deoxycytidine should result in an uptake of 40 pmol/10 s of deoxycytidine and 49 pmol/10 s of uracil, based on uracil uptake (Fig. 2) and deoxycytidine uptake in the presence of tetrahydrodridine and glucose (Fig. 3). Observed uptake is 41 pmol/10 s of deoxycytidine and 54 pmol/10 s of uracil (Table III). Uracil uptake appears to be inhibited completely at higher deoxycytidine concentrations by the high intracellular uracil concentration resulting from deoxycytidine degradation, since total uptake becomes proportional to the 5-s deoxycytidine concentration.

Deoxycytidine Uptake in the Presence of Tetrahydrodridine—Fig. 3 compares deoxycytidine uptake in E. coli B and a showdomycin-resistant mutant at varying substrate concentrations. Uptake in E. coli B is stimulated by 5 mM glucose, particularly at lower substrate concentrations, and inhibited by cytidine or deoxyadenosine. Uptake follows Michaelis-Menten kinetics, both in the presence and absence of glucose, and Lineweaver-Burk plots are linear. In the showdomycin-resistant mutant, uptake of deoxycytidine in the presence of glucose and tetrahydrodridine is very low as compared to the parent strain, and is linear with increase in substrate concentration up to 1 mM substrate.

Kinetic data for deoxycytidine uptake in E. coli B are given in Table V. The Km for deoxycytidine uptake in the absence of glucose is almost 10-fold higher than in the presence of glucose, but the Vmax is unchanged. A possible explanation for this finding will be presented in the discussion.
TABLE VI

Effect of heterologous nucleosides on deoxycytidine uptake by Escherichia coli B and a showdomycin-resistant mutant

Uptake was measured by the standard assay, with addition of 205 μM H₃Urd and 5 mM glucose. For E. coli B, deoxycytidine concentration was 0.5 μM and inhibitor concentration, 3 μM. For the showdomycin-resistant mutant, deoxycytidine concentration was 40 μM and inhibitor concentration, 250 μM. Time of incubation was 10 s for E. coli B and 1 min for the showdomycin-resistant mutant.

| Cell strain                  | Uptake with inhibitor addition (%) | Uptake with inhibitor addition (nmol / 10 s) |
|-----------------------------|-----------------------------------|--------------------------------------------|
|                             | None                              | Cytidine | Adenosine | Uracil | Deoxycytidine | Guanosine |
| E. coli B                   | 100                               | 33.8     | 20.4      | 32.0   | 29.8          | 100       |
| (0.06)*                     |                                   |          |           |        |                |           |
| Showdomycin-resistant mutant| 100                               | 100      | 99.8      | 98.6   | 99.2          | 100       |
| (0.006)*                    |                                   |          |           |        |                |           |

* Uptake in nanomoles.

TABLE VII

Effect of energy poisons on deoxycytidine uptake

Uptake was assayed by the standard procedure, with the addition of 5 mM glucose and 205 μM H₃Urd, except that when arsenate was to be added potassium arsenate was substituted for potassium phosphate in Medium A. This modified buffer was used in the final wash and the incubation medium. KCN concentration was 20 mM, and was added just prior to preincubation. Deoxycytidine concentration was 0.5 μM.

| Additions to incubation | dCyd uptake | Uptake |
|-------------------------|-------------|--------|
|                         | nmol / 10 s | %      |
| none                    | 0.06        | 100    |
| 62 mM arsenate          | 0.0024      | 4      |
| 20 mM KCN               | 0.0013      | 2.2    |
| 62 mM arsenate, 20 mM KCN| 0          | 0      |

Cytidine and deoxyadenosine inhibit deoxycytidine uptake competitively in the presence of glucose, while guanosine has no effect. Concentrations of both substrate and inhibitors after 5-s incubation were calculated and used in all kinetic determinations, since the initial substrate concentration decreases by 10% at lower concentrations during the 10-s incubation period.

Table VI shows that uridine and adenosine also inhibit deoxycytidine uptake in E. coli B. Kinetic data for inhibition by these nucleosides were not determined, since they are rapidly degraded both intracellularly and in the periplasmic space, whereas cytidine degradation is prevented by tetrahydrouridine, and deoxyadenosine degradation is slow. Heterologous nucleosides do not inhibit uptake in the mutant.

Effect of energy poisons on deoxycytidine uptake in E. coli B—Addition of either potassium arsenate or KCN greatly reduces uptake of deoxycytidine in the presence of exogenous glucose (Table VII). The uptake in the presence of these inhibitors is one-half and one-fifth, respectively, of that in the absence of glucose without the addition of an energy poison. Addition of both arsenate and KCN eliminates uptake.

DISCUSSION

The characteristics of deoxycytidine transport in E. coli B show that tetrahydrouridine does not enter the cell. Lack of a kinase for deoxycytidine has been reported previously (13), and we have failed to find any conversion of deoxycytidine to the nucleotide level in a cell-free system. Therefore, recovery of intracellular radioactivity in the nucleotide fraction, as well as in uracil and uridine fractions, can be accounted for only by prior deamination to deoxyuridine. The fraction of total uptake represented by nucleotides, uridine, and uracil is the same in the presence and absence of tetrahydrouridine. It is therefore apparent that tetrahydrouridine does not inhibit intracellular deamination of deoxycytidine.

Prior to the discovery of periplasmic cytidine deaminase (4) and nucleoside phosphorylase, it was assumed by other authors (3, 14) that uracil found in the medium is formed intracellularly and is subsequently excreted. The data of Table II show that this assumption is incorrect, since tetrahydrouridine greatly reduces the amount of uracil recovered in the medium, even when intracellular uridine and uracil concentrations are considerably higher than those found in the absence of the deaminase inhibitor. The concept that uracil in the medium is the result of periplasmic deamination to deoxyuridine, followed by periplasmic cleavage to uracil and deoxyribose 1-phosphate is also supported by the data in Table III. Comparison of transport data from [2-14C]- and [U-14C]deoxycytidine shows that the intracellular pyrimidine moiety concentration is higher than the intracellular deoxyribosyl moiety concentration when tetrahydrouridine is not present. This indicates that uracil, formed in the periplasmic space, is transported into the cell without accompanying deoxyribose transport. The deaminase inhibitor prevents this periplasmic degradation as evidenced by the equal uptake of the pyrimidine and deoxyribosyl moieties (Table III). This confirms the conclusion stated previously ("Results") that the small amount of uracil found in the medium when tetrahydrouridine is present must result principally from a very slow rate of periplasmic degradation of deoxycytidine.

Although equality of the intracellular pyrimidine and deoxyribosyl moieties when tetrahydrouridine is present indicates transport of deoxycytidine before cleavage to base and sugar, it does not preclude entry into the cell as a nucleotide, by a group translocation mechanism. In this case the high deoxycytidine concentration found intracellularly could be the result of dCMP breakdown by phosphatases. However, transport as dCMP cannot occur since a kinase for deoxycytidine is not present in E. coli B. Nucleotide formation requires prior deamination to deoxyuridine. Since, in the presence of tetrahydrouridine, this reaction in the periplasmic space is largely inhibited, while total uptake is generally greater, the possibility of group translocation of deoxycytidine as DUMP is eliminated. It can be concluded that in the presence of tetrahydrouridine, deoxycytidine is transported as the intact nucleoside, and its accumulation against a gradient occurs without interference by the analog.

Kinetic data for deoxycytidine transport in the presence of tetrahydrouridine indicate the existence of a single mode of transport that is stimulated by an energy source. We have found no evidence of the second transport system proposed by Komatsu and Tanaka (14, 15) in E. coli K12 that is retained by their showdomycin-resistant mutant. Lineweaver-Burk plots of uptake are linear in E. coli B, and our showdomycin-resistant mutant appears to have lost the ability to transport deoxycytidine by a mediated process. The stimulatory effect of glucose is consistent with an active transport system in E. coli B, as is the intracellular accumulation of nucleoside to give a 40:1 concentration gradient (Table II). Inhibition of transport by energy poisons

* Unpublished data.
is also suggestive of an energy requirement for transport (Table VII).

In the absence of glucose, the \( K_m \) for deoxycytidine transport is increased, but the \( V_{\text{max}} \) remains the same. This can be taken as evidence that the transport system can function in the absence of an energy source, but with a decreased affinity for substrate. The energy source would then act only on lowering the \( K_m \) without increasing \( V_{\text{max}} \). Kaback and Hong (16) have published similar findings for amino acid transport by \( E. coli \).

In our showdomycin-resistant mutant, transport of deoxycytidine in the absence of glucose is too low to be measured. Glucose increases uptake in the mutant, but it is still very low compared with transport in the wild type. This residual transport probably represents slow, passive diffusion of deoxycytidine, with glucose stimulation explained by increased conversion to nucleotides. This view is supported by the finding (Table VI) that none of the heterologous nucleosides that inhibit uptake in \( E. coli \) B has any effect on deoxycytidine uptake in the mutant.

Uracil transport is also energy-dependent. The ratio of intracellular uracil concentration to extracellular uracil concentration can be 90:1, indicating an active transport for uracil. Part of the energy requirement can be accounted for, as in the case of deoxycytidine, by conversion to the nucleotide level. However, intracellular nucleotides cannot result from group translocation involving a uracil phosphoribosyltransferase, as has been reported in \( E. coli \) K12 and \( E. coli \) EA1 (4). In agreement with the results of Crawford et al. (10), we were unable to detect any uracil phosphoribosyltransferase activity in extracts of \( E. coli \) B, although high levels of adenine phosphoribosyltransferase were found. In addition, the \( K_m \) of 0.5 \( \mu \)M for uracil transport in \( E. coli \) B is very much lower than the \( K_m \) of 43 \( \mu \)M reported for uracil uptake in \( E. coli \) K12 (4). It is therefore unlikely that the high intracellular uracil concentrations result from nucleotide breakdown. Uracil is probably transported as the free base in \( E. coli \) B, although group translocation of uracil as uridine cannot be ruled out since uridine is also present intracellularly at relatively high concentration.

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