Concentrative Uridine Transport by Murine Splenocytes: Kinetics, Substrate Specificity, and Sodium Dependency

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ABSTRACT

A previous report from this laboratory indicated that the concentration of free uridine (Urd) in many normal murine tissues greatly exceeds that in plasma. We now report that Urd uptake by isolated murine splenocytes is concentrative, and that the rate of uptake from medium containing 10 to 500 μM [3H]Urd conforms to a process that is saturable with a K_m of 38.0 ± 4.1 (SE) μM and V_max of 2.70 ± 0.27 pmol/s/μl cell water. Other ribosyl and deoxyribosyl pyrimidine nucleosides or their analogues were not concentrated by splenocytes; however, ribosyl and deoxyribosyl purine nucleosides and, to a lesser extent, deoxyuridine did inhibit Urd uptake. In this system Urd uptake was not inhibited by 1 μM nitrobenzylthioinosine or 10 μM dipyridamole but was significantly inhibited by 5 mM NaNO_3 or 250 mM KCN. Transport of Urd involves Na^+ cotransport as evidenced by complete inhibition when Na^+ is replaced by Li^+ in the incubation medium, and it is also inhibited by 3 mM ouabain. Active Urd transport coexists with the nonspecific, carrier mediated, facilitated diffusion of nucleosides as demonstrated by the inhibition of Urd efflux and thymidine influx in splenocytes by nitrobenzylthioinosine and dipyridamole.

INTRODUCTION

The nonconcentrative transport of nucleosides across the cell membrane has been extensively studied over the past 15 years (1–4). Using mammalian erythrocytes (1, 2, 5, 6) and a variety of neoplastic cell lines (3, 7) as model systems, several groups have established this process to be carrier mediated facilitated diffusion with the carrier system having broad specificity toward both purine and pyrimidine ribose and deoxyribose nucleosides.

Studies by Belt (8, 9) and others (10, 11) suggest that there may be several nonconcentrative nucleoside transport systems operational in cells which can be distinguished by their sensitivity to nucleoside transport inhibitors such as NBMPR, dipyridamole, and p-chloromercuribenzenesulfonate (8, 12–14). Jarvis and Young (15) and Young et al. (7, 16) using NBMPR binding activity as a probe, have partially purified and characterized this carrier molecule from human erythrocyte membranes and report that it is a band 4.5 polypeptide with a molecular weight of 45,000–65,000.

In addition to the facilitated diffusion of nucleosides, reports by Kuttetsch and Nelson (17), Kuttetsch et al. (18), and Trimble and Coulson (19) indicate that mammalian kidney epithelial cells possess an active transport system which is responsible for the secretion of deoxyadenosine and the reabsorption adenine. More recently, Schwenk et al. (20) as well as LeHir and Dubach (21) have presented data indicating that guinea pig intestinal epithelial cells and vesicles prepared from rat kidney epithelial cells possess a Na^+ dependent Urd transport system which functions to transport Urd into cells against a concentration gradient. Spector and Huntoon (22) have also reported that nucleoside uptake in choroid plexus is energy dependent but that the efflux from this system is inhibited by NBMPR.

Our interest in nucleoside transport, and more specifically Urd transport, derives from our observation that in vivo the utilization of radiolabeled Urd for nucleotide and nucleic acid biosynthesis in tissues is generally insensitive to large increases in the plasma pool of Urd (23). Further investigation of this phenomenon has revealed that tissues contain pools of free Urd up to thirteenfold greater than the concentration of Urd in plasma (24). While the size of tissue Urd pools may relate to the Urd phosphorylation activity, the pools themselves appear to result from a concentrative transport process. Using suspensions of murine splenocytes, we now report the transport kinetics, substrate specificity, and Na^+ dependency of this uptake process in these nonepithelial cells. Preliminary aspects of these findings have been recently reported (25).

MATERIALS AND METHODS

Animals. All experiments utilized 3- to 6-month old C57BL/6 female mice (hereafter called C57) obtained from NIH.

Chemicals. Urd, dTThd, dUrd, dFdUrd, uracil, (5-methyl)Urd, and dThd, were purchased from Sigma (St. Louis, MO). [^{6-3H}]Urd (28 Ci/mmol), and [^{6-3H}]FdUrd (18 Ci/mmol), [^{6-3H}]uracil (22 Ci/mmol), [^{5-3H}]cytidine (28 Ci/mmol), and [^{5-3H}]dThd (18 Ci/mmol) were purchased from New England Nuclear (Boston, MA). [COCO²H]inulin (10 mCi/mmol) was purchased from Amersham (Arlington Heights, IL) while [^{2-3H}]inosine (25 Ci/mmol), [^{6-3H}]Urd (18 Ci/mmol), [^{5-3H}]TThd (18 Ci/mmol), [^{6-3H}]uracil (22 Ci/mmol), [^{5-3H}]cytidine (28 Ci/mmol), and [^{5-3H}]FdUrd (18 Ci/mmol), and [^{5-3H}]dThd (18 Ci/mmol) were purchased from Moravek (Brea, CA). Fischer’s medium was purchased from Gibco (Grand Island, NY). All other reagents were of analytical grade and purchased from Baker Chemicals.

Splenocyte suspensions were prepared from C57 mice sacrificed by cervical dislocation. The spleen was quickly removed and dispersed into 4 ml of Fischer’s medium (pH 7.4) at 37°C by gently abrading the spleen between two frosted microscope slides as previously described (24). After allowing the tissue debris to settle, the cell density was adjusted to 1–2 x 10⁷ cells/ml by the addition of fresh medium. The splenocytes were then centrifuged at 100 x g for 10 minutes and washed twice in plasma (24). While the size of tissue Urd pools may relate to the Urd phosphorylation activity, the pools themselves appear to result from a concentrative transport process. Using suspensions of murine splenocytes, we now report the transport kinetics, substrate specificity, and Na^+ dependency of this uptake process in these nonepithelial cells. Preliminary aspects of these findings have been recently reported (25).
"rapid sampling tube" consisting of a 400-μl Eppendorf microfuge tube containing 120 μl of oil (a 16:84 mixture of Fisher 0121 light paraffin oil and Dow Corning 350 silicone fluid having a specific gravity of approximately 1.04 g/ml) layered over 35 μl of 15% trichloroacetic acid. The splenocytes were removed from the medium and pelleted into the trichloroacetic acid by centrifugation for 2 min at 10,000 × g in a Beckman Model B microfuge. The microfuge tubes were frozen and then cut through the oil layer and each portion placed in a plastic minicentistillation vial containing 0.2 ml H2O and vortexed vigorously. Four ml of Liquiscint (National Diagnostic, Somerville, NJ) were then added, and the radioactivity in the cell pellet resulting from each isotope was determined and used to calculate the intracellular ([3H-related]-[4C-related] dpm) and trapped medium ([4C-related] dpm) volume of the cell pellet (24, 26).

The kinetics of Urd uptake were determined by mixing with a dual syringe dispenser (26), 100 μl of the splenocyte suspension with an equal volume of medium containing concentrations of [3H]Urd ranging from 10 (4 μCi/ml)–500 μM (50 μCi/ml) in the rapid sampling tubes described above. At various times after mixing (ranging from 2 s, the earliest time point from which reliable data can be obtained due to the finite period of time it takes cells to pellet through oil, to 10 min) the cells were pelleted and the intracellular concentration of [3H]Urd calculated from the radioactivity in the cell pellet. To insure that the cell pellet associated cpm were Urd and not Urd metabolities cell pellets were also analyzed by HPLC. Pooled cell pellet fractions from identical time points were homogenized and then extracted with an equal volume of n trioctylamine in Freon. The [3H]Urd content of these samples was determined by reverse-phase HPLC methods previously reported (23). Under all conditions used, over 90% of the intracellular radioactivity eluted with authentic Urd during the first 2 min of incubation in 50 μM [3H]Urd. Using these data, the initial rate of [3H]Urd transport at each media concentration of Urd tested was estimated using only early time points (at least three where possible) where transport was linear and in all cases did not include data generated after 40 s of incubation. The Kₘ and Vₘₐₓ were calculated from this data by Line- weaver-Burke analysis using the Calc-Star program.

The substrate specificity of this transport process was assayed by substituting tritiated uracil, deoxyuridine, thymidine, cytidine, FdUrd, and inosine at 5 μM and 2 μCi/ml for [3H]Urd. To determine if other related compounds could compete with [3H]Urd for transport, 100 μl of splenocyte suspension were mixed with an equal volume of 100 μM [3H]Urd (20 μCi/ml) containing either 100 or 1000 μM competing compound in rapid sampling tubes and the velocity of [3H]Urd uptake determined as described above.

The sensitivity of concentrative Urd transport to inhibitors was assessed by incubating splenocytes in Fischer's medium containing 5 mM NaN₃, 250 μM KCN, 3 mM ouabain, 1 μM NBMPR, or 10 μM dipryridamole for 15 min at 37°C. Additional studies were conducted by incubating splenocytes in Na⁻′free Hanks' balanced salt solution (Gibco) in which NaCl was replaced with LiCl and containing 6 mM glucose, 4 mM HEPES (pH 7.4) and 1 μM NBMPR. One hundred μl of the resulting cell suspensions were mixed with an equal volume of medium containing either 100 μM [3H]Urd (20 μCi/ml) or 100 μM [3H]Tdh (20 μCi/ml) in a rapid sampling tube and processed as above. The [3H]Urd content of cell pellet samples was confirmed by the HPLC method referred to above. The [3H]Tdh content of cell pellets was confirmed by using a modification of previously reported reverse-phase HPLC methods (27) utilizing a Rainsian C-18 microsorb column maintained at 15°C and eluted at 1 ml/min with 10 mM phosphoric acid containing 1 mM heptane sulfonic acid (pH 3.2). Under these conditions the retention time for Tdh was 39 min. In all spleocyte experiments, after incubating in 50 μM [3H]Tdh, not less than 85% of the intracellular radioactivity was associated with Tdh during the first 2 min.

The relationship between extracellular Na⁺ and nucleoside transport was studied by dispersing spleen cells into either Hanks' balanced salt solution containing 6 mM glucose and 4 mM HEPES (pH 7.4) or into Na⁻′free Hanks' balanced salt solution (containing 0.14 M LiCl instead of 0.14 M NaCl) with 6 mM glucose and 4 mM HEPES (pH 7.4). After incubating for 15–20 min at 37°C, [3H]Urd and [3H]Tdh transport was assayed as described above. Additional studies were conducted by suspending splenocytes from the same population of cells directly in Hanks' balanced salt solution containing 6 mM glucose and 4 mM HEPES (pH 7.4) at 37°C or Na⁻′free Hanks' balanced salt solution containing 0.14 M LiCl plus 6 mM glucose and 4 mM HEPES (pH 7.4) at 37°C and measuring [3H]Urd transport under each condition. To assess the zero-trans rate of [3H]Urd uptake, splenocytes were depleted of their intracellular pools of Urd by incubation for 15 min at 37°C in Na⁻′free Hanks' balanced salt solution containing 0.14 M LiCl, 4 mM HEPES, and 6 mM glucose (HPLC analysis revealed that within this time period intracellular Urd pools were more than 90% depleted). NBMPR (5 μM) was then added, the cells pelleted by centrifugation at 200 × g for 5 min and then resuspended in Hanks' balanced salt solution (0.14 M NaCl) containing 4 mM HEPES, 6 mM glucose, and 5 μM NBMPR (pH 7.4) at 37°C. Urd uptake was assayed by mixing 100 μl of cell suspension with an equal volume of Hanks' balanced salt solution (0.14 M NaCl) containing [3H]Urd at concentrations ranging from 10 (2 μCi/ml)–300 μM (40 μCi/ml).

The efflux of Urd from splenocytes was studied by "loading" cells with [3H]Urd by incubation for 5 min at 37°C in Hanks' balanced salt solution (0.14 M NaCl) containing 4 mM HEPES (pH 7.4), 6 mM glucose, and 50 μM [3H]Urd (10 μCi/ml). The cell suspension then was divided into two aliquots and to one was added 5 μM NBMPR. Cells in both suspensions were pelleted by centrifugation and resuspended in Hanks' balanced salt solution (0.14 M NaCl) containing 4 mM HEPES (pH 7.4) and 6 mM glucose at 37°C. For the cells previously exposed to NBMPR, 5 μM NBMPR were also added to the resuspension medium. At various times 200 μl of cell suspension were placed in rapid sampling tubes and processed as described above with the identity of the radioactivity associated with the cell pellet confirmed by HPLC analysis.

Urd transport in L1210 cells was studied in cells grown in suspension cultures in Fischer's medium containing 10% fetal bovine serum. Cells were harvested in exponential growth and resuspended at approximately 5 × 10⁶ cells/ml in Fischer's medium containing no serum. The effect of NBMPR, NaN₃, or the removal of Na⁺ from the incubation medium on the ability of these cells to transport [3H]Urd (50 μM; 10 μCi/ml) was determined as described above for splenocytes. Unlike splenocytes, however, HPLC analysis of L1210 cell pellets for intact [3H]Urd revealed that when these cells were exposed to 50 μM [3H]Urd only 30–40% of the intracellular radioactivity was associated with Urd after 2 min of incubation and, therefore, all Urd transport data were calculated based on data from HPLC analysis.

RESULTS

We have previously reported that dispersed splenocytes are capable of concentrating [3H]Urd from the incubation medium (24). The kinetics of [3H]Urd uptake by splenocytes was studied by incubating these cells in medium containing [3H]Urd at concentrations ranging from 10–500 μM (Fig. 1). The initial (maximal) velocity of [3H]Urd uptake as a function of [3H]Urd concentration displays saturation kinetics with an apparent Kₘ of 38.0 ± 4.1 (SE) μM and a Vₘₐₓ of 2.70 ± 0.27 pmol/s/μl cell water (r = 0.98) (Fig. 2).

Incubating murine splenocytes with a variety of 3H-labeled pyrimidine nucleosides or nucleoside analogues (Table 1) demonstrated that none of these closely related compounds was concentrated by splenocytes under these assay conditions. Furthermore, with the exception of dUrd, the uptake of [3H]Urd was not significantly inhibited by other pyrimidine nucleosides. Several purine ribonucleosides, and to a lesser degree deoxyribo-nucleosides, however, were potent inhibitors of [3H]Urd uptake, and the data in Table 1 suggest that [3H]inosine may also be concentrated in this system.

Concentrative Urd uptake was not inhibited by 1 μM NBMPR (Fig. 3) or 10 μM dipryridamole (data not shown), compounds that inhibit the facilitated diffusion of nucleosides across cell membranes.
CONCENTRATIVE Urd TRANSPORT

Fig. 1. Concentrative [3H]Urd transport in murine splenocytes. Splenocytes (1-2 x 10^7 cells/ml) were rapidly mixed with 10 (Δ), 50 ( ), or 150 μM (○) [3H]Urd for various times and harvested as described in "Materials and Methods." The intracellular [3H]Urd concentration was calculated from the cell pellet associated cpm and confirmed by HPLC analysis. Points, mean ± SE (bars) of 6-14 determinations and for clarity, data from the 5- and 15-s time points are not included.

Fig. 2. Kinetics of [3H]Urd transport in murine splenocytes. At each media concentration of [3H]Urd tested, the initial rate of [3H]Urd transport was calculated using only early time points where transport was linear and regardless of the media concentration of Urd did not include data generated after 40 s of incubation. The apparent Km (38.0 ± 4.1 μM) and Vmax (2.7 ± 0.27 pmol/s/μl of cell water) were obtained by regression analysis of the Lineweaver-Burke plot (correlation coefficient >0.98) using the Calc-Star statistical program. Points, mean ± SE (bars) of 6-10 determinations.

membranes (12, 28). However, in the same population of splenocytes, these compounds effectively inhibited the nonconcentrative uptake of 50 μM [3H]dThd. The dual nature of Urd transport in these cells was made more apparent when splenocytes were incubated in Na+-free medium. Under these conditions, the Km for Urd decreased from approximately 30 μM to less than 3 μM, and these cells were resuspended in Urd-depleted medium revealed that Urd efflux from splenocytes is inhibited by NBMPR (Fig. 5). Thus, replaced by LiCl resulted in the loss of their ability to concentrate Urd (Fig. 3). When Na+-depleted cells were resuspended in medium containing a physiological concentration of Na+ the concentrative uptake of Urd was completely restored (Fig. 4).

Incubating splenocytes in media containing 3 mM ouabain also inhibited the concentrative uptake of Urd by nearly 50% despite the relatively poor sensitivity of murine Na+-K+-ATPases to this inhibitor (30). Removing Na+ from the incubation medium had no affect on the nonconcentrative uptake of 50 μM [3H]dThd in the same population of cells (Fig. 3). Similarly, incubating L1210 cells in Na+-free media did not affect the facilitated diffusion of [3H]Urd in this leukemia cell line (Fig. 3).

To preclude the possibility that intracellular pools of free Urd were influencing the uptake of Urd in these cells the initial velocity of Urd transport in Urd-depleted cells was determined. Cells were depleted of intracellular Urd by incubation in Na+-free medium. After 15 min, the intracellular concentration of free Urd decreased from approximately 30 μM to less than 3 μM, and these cells were resuspended in Na+-containing medium with 5 μM NBMPR to inhibit the facilitated diffusion of nucleosides. Under these less physiological conditions, the Km for Urd uptake was 60 μM and the Vmax was 2.4 pmol/s/μl, values similar to those determined in normal splenocytes.

Analysis of the intracellular concentration of [3H]Urd in splenocytes "loaded" by incubation in 50 μM [3H]Urd for 5 min and then resuspended in Urd-free medium revealed that Urd efflux from splenocytes is inhibited by NBMPR (Fig. 5). Thus,

Table 1 Substrate specificity of concentrative nucleoside uptake by murine splenocytes

<table>
<thead>
<tr>
<th>Initial velocity of [3H]Urd uptake (pmol/s/μl cell water) at competitor concentration of</th>
<th>pooled substrate</th>
<th>50 μM</th>
<th>500 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ( [3H]Urd alone)</td>
<td>1.51 ± 0.14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Competing compound

Pyrimidine nucleosides

| Uridine | 0.92 ± 0.11 | 0.38 ± 0.13 |
| Decoxuridine | 1.17 ± 0.28 | 0.56 ± 0.15 |
| 5-Methyl uridine | ND | 1.66 ± 0.25 | 0.79 ± 0.44 |
| Thymidine | 1.70 ± 0.23 | 0.69 ± 0.10 |
| Cytidine | 1.71 ± 0.21 | 0.96 ± 0.18 |
| Deoxycytidine | ND | 1.51 ± 0.35 | 1.65 ± 0.35 |
| 5-Fluorouridine | 1.46 ± 0.34 | 0.81 ± 0.16 |
| 5-Fluorodeoxyuridine | 0.9 ± 0.3 | 1.64 ± 0.33 | 1.44 ± 0.29 |

Purine nucleosides

| Adenosine | 0.56 ± 0.13 | 0.13 ± 0.05 |
| Deoxyadenosine | ND | 0.81 ± 0.23 | 0.36 ± 0.13 |
| Guanosine | ND | 0.91 ± 0.09 | 0.16 ± 0.11 |
| Deoxyguanosine | ND | 1.27 ± 0.19 | 0.40 ± 0.15 |
| Inosine | ND | 0.71 ± 0.12 | 0.15 ± 0.07 |
| Deoxyinosine | ND | 0.90 ± 0.10 | 0.26 ± 0.10 |

Other compounds

| Uracil | 1.1 ± 0.4 | 1.55 ± 0.01 | 1.59 ± 0.48 |
| Hypoxanthine | ND | 1.69 ± 0.18 | 1.66 ± 0.15 |
| Arabinosyl uracil | ND | 1.36 ± 0.20 | 1.21± |
| Nitrobenzylthiouracile | ND | 1.53 ± 0.20 | 1.51± |
| Glucose | ND | 1.75 | 1.63 |

ND, not determined.

* Average of two determinations.

Table 2 Substrate specificity of concentrative nucleoside uptake by murine splenocytes

<table>
<thead>
<tr>
<th>Km (μM)</th>
<th>Km</th>
<th>Km</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urd</td>
<td>38.0 ± 4.1</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>Uridine</td>
<td>4.4 ± 0.9</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>Decoxuridine</td>
<td>0.9 ± 0.3</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>5-Methyl uridine</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Thymidine</td>
<td>1.0 ± 0.2</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>Cytidine</td>
<td>0.8 ± 0.2</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>Deoxycytidine</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5-Fluorouridine</td>
<td>1.3 ± 0.3</td>
<td>1.2 ± 0.7</td>
</tr>
<tr>
<td>5-Fluorodeoxyuridine</td>
<td>0.9 ± 0.3</td>
<td>1.64 ± 0.33</td>
</tr>
</tbody>
</table>

[ND, not determined.
In all experiments 10 μM dipyridamole had essentially the same effect as 1 mM NaN₃ and 250 μM KCN had essentially the same effect as 5 mM NaN₃. The nucleoside and the uptake compared to uptake in the absence of inhibitor (control, *). The nucleoside was assayed under these conditions as described in "Materials and Methods." Points, mean ± SE (bars) of 4-16 determinations.

Fig. 3. Effect of inhibitors on Urd and dThd transport in splenocytes and L1210 cells. Splenocytes (1-2 × 10⁷ cells/ml) were incubated at 37°C in either Hanks’ balanced salt solution containing 6 mM glucose and 4 mM HEPES (pH 7.2) containing 1 μM NBMPR (△) or 5 mM NaN₃ (△) or in Na*-free Hanks’ balanced salt solution (LiCl replaced NaCl) containing 6 mM glucose and 4 mM HEPES (pH 7.2) with (*) or without (△) 1 μM NBMPR. After 15 min [³H]Urd or [³H]dThd transport was assessed as described in "Materials and Methods." Points, average of two determinations.

For clarity the dipyridamole and KCN data have not been included. Points, mean ± SE (bars) of four determinations.

Fig. 4. Sodium dependency of concentrative Urd transport in murine splenocytes. Splenocytes (1 × 10⁷ cells/ml) were dispersed at 37°C into either Hanks’ balanced salt solution containing 6 mM glucose and 4 mM HEPES (pH 7.2) or Na*-free Hanks’ balanced salt solution (LiCl replaced NaCl) containing 6 mM glucose or 4 mM HEPES (pH 7.2). [³H]Urd transport was quantitated in each condition by incubation in medium containing 50 μM [³H]Urd as described in "Materials and Methods." Points, average of two determinations.

In contrast to the systems responsible for the facilitated diffusion of nucleosides, the active transport of Urd by splenocytes exhibits a considerable specificity with none of the other pyrimidine nucleosides assayed under these conditions appearing to serve as substrates. However, preliminary experiments...

Fig. 5. Effect of NBMPR on the efflux of [³H]Urd from murine splenocytes. Splenocytes were "loaded" with [³H]Urd by incubation for 5 min in medium containing 50 μM [³H]Urd (10 μCi/ml) and then resuspended in Urd-free medium containing 5 μM NBMPR (△). After resuspension, the intracellular [³H]Urd content was determined as described in "Materials and Methods." Control incubations (C) were conducted in the absence of NBMPR. Points, mean ± SE (bars) of four determinations.

Urd entry is predominately an energy dependent system, but its efflux is largely the result of the facilitated diffusion mechanism.

DISCUSSION

The ability of freshly dispersed murine splenocytes to concentrate [³H]Urd sharply contrasts with the mechanism responsible for the facilitated diffusion of nucleosides extensively studied in a variety of neoplastic cell lines and erythrocytes (1, 2, 5, 7). This concentrative transport system, having high substrate specificity, apparently requires the cotransport of Na⁺ and thus is similar to that reported to exist for glucose (31, 32) and amino acids in various epithelial cells (33, 34). There was a possibility that the concentrative uptake of [³H]Urd could reflect a "Urd binding protein" in the cytosol of these cells. However, extracts of murine spleen mixed with high specific activity [³H]Urd and chromatographed on a Bio-Gel-60 column revealed that the elution profile of [³H]Urd was not affected by cytosolic proteins. It was also possible that large intracellular pools of free Urd in freshly isolated splenocytes could "recruit" transport molecules and "drive" the uptake of [³H]Urd in a manner similar to that reported for adenine in L1210 cells (35). However, the kinetics of the zero-trans uptake of [³H]Urd in splenocytes suggests that this is not the case. The K₅₀ for Urd in the NBMPR sensitive facilitated diffusion system mother cell types ranges between 210 and 450 μM (6, 36, 37), values 6- to 10-fold higher than what we report for the concentrative system in splenocytes (38 μM). The V₅₀ for the active transport of Urd (2.7 pmol/s/μl cell water) is low but within the range of values reported for the facilitated diffusion of Urd (10, 37-39). Thus, the high intracellular Urd pools retained in splenocytes can be explained, in part, by the somewhat lower V₅₀ of facilitated diffusion in splenocytes, the low potential of these cells to metabolize Urd (24), and the greater affinity of Urd for the active transport system. It is important to recognize that both nucleoside transport systems coexist in these and probably other cell types and that the ultimate internal nucleoside concentration reflects their algebraic sum as modified by intracellular metabolism.

In contrast to the systems responsible for the facilitated diffusion of nucleosides, the active transport of Urd by splenocytes exhibits a considerable specificity with none of the other pyrimidine nucleosides assayed under these conditions appearing to serve as substrates. However, preliminary experiments...
utilizing [H]inosine as substrate suggest that purine nucleoside transport in splenocytes may also be a Na+-dependent process despite the fact that intracellular concentrations of these nucleosides, as measured by HPLC, do not greatly exceed those transport in splenocytes may also be a Na+-dependent process utilizing [3H]inosine as substrate suggest that purine nucleoside carrier protein.

explain the efficiency with which large doses of Urd reverse in the medium (Table I).4 Our present findings contrast with despite the fact that intracellular concentrations of these nu in intracellular concentrations of Urd in vivo may be inoperative. Alternatively, the active transport of Urd may be lost as cells are adapted to growth in culture. However, we have recently detected concentrative Urd transport in the T-cell subpopulation of murine splenocytes up to 23 days after dispersion in serum supplemented medium. In progress are studies to determine if concentrative Urd transport is lost as these cells continue to adapt to long term culture driven by interleukin 2.

The ability of cells in normal host tissue to generate and sustain high intracellular concentrations of Urd in vivo may explain the efficiency with which large doses of Urd reverse FUra-induced toxicity (40, 41). These findings further suggest that by carefully controlling plasma Urd levels with agents such as 5-benzylacyclouridine (5-benzyl-1-[2'-hydroxyethoxymethylj uracil) one may achieve increases in the therapeutic effective-ness of Na-phosphoracetyl)-l-aspartate (PALA) (42), pyrazofurin (43), and FUra. Indeed, our recent report (23) of an increase in the therapeutic effectiveness of FUra by its use in combination with 5-benzylacyclouridine may be a case in point. In addition to the therapeutic implications, we are also exam-ining the ability of nucleoside analogues to inhibit the active transport of Urd with the hope of identifying selective inhibitors that may be useful in the isolation and characterization of the carrier protein.

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