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Hypoxanthine Transport in Mammalian Cells: Cell Type-Specific Differences in Sensitivity to Inhibition by Dipyridamole and Uridine

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Summary. We have measured by rapid kinetic techniques the zero-*trans* influx of hypoxanthine in various cell lines and its sensitivity to inhibition by uridine, dipyridamole, nitrobenzylthioinosine and nitrobenzylthiopurine. The results and those reported earlier divided the cells into two distinct groups. In mouse P388, L1210 and L929 cells uridine and hypoxanthine had little effect on the transport of each other, supporting the view that nucleosides and hypoxanthine are transported by different carriers. In these cells, hypoxanthine transport was also uniquely resistant to inhibition by dipyridamole (IC_{50} (50% inhibition dose) > 30 μM). In Novikoff and HTC rat hepatoma, Chinese hamster ovary and Ehrlich ascites tumor cells, on the other hand, hypoxanthine and uridine inhibited the transport of each other about 50% at a concentration corresponding to the Michaelis-Menten constant of their transport, and hypoxanthine transport was strongly inhibited by dipyridamole (IC_{50} = 100 to 400 nM). Although these results are compatible with the view that nucleosides and hypoxanthine are transported by a common carrier in these cells, this conclusion is not supported by the finding that uridine transport is strongly inhibited in some of these cell lines, as in the first group of cells, by nitrobenzylthioinosine, whereas hypoxanthine transport is highly resistant in all cell lines tested. In contrast, the transport of both substrates is highly resistant to inhibition by nitrobenzylthiopurine. The Michaelis-Menten constants for uridine transport are about the same in all cell lines. The Michaelis-Menten constants for hypoxanthine transport are similar to those for uridine transport in some cell lines, but are much higher in others. This difference is unrelated to the sensitivity of uridine and hypoxanthine transport to inhibition by each other or dipyridamole.

Key Words transport · hypoxanthine · uridine · cultured animal cells · dipyridamole

Introduction

The Michaelis-Menten constants and maximum velocities for the transport of nucleosides and Hyp are of the same order of magnitude for some different lines of cultured mammalian cells (Plagemann & Wohlhueter, 1980, 1982). In addition, both nucleoside and Hyp transport across the plasma membrane are indifferent with respect to direction (directional symmetry) and the mobilities of substrate-loaded and empty carrier are equivalent (transmembrane equilibration of substrate under equilibrium exchange and zero-*trans* conditions is the same). We have also found that nucleosides strongly inhibit the transport of Hyp in Novikoff rat hepatoma cells, and, vice versa, that Hyp similarly inhibits the transport of nucleosides in these cells (Marz, Wohlhueter & Plagemann, 1979; Wohlhueter, Marz & Plagemann, 1979; Plagemann & Wohlhueter, 1980). A similar finding has been reported for CHL cells (Slaughter & Barnes, 1979; Slaughter, Fenwick & Barnes, 1981). Because Hyp and dThd inhibited the transport of each other in an apparent competitive manner with K_i values similar to the K_m values for their transport, Slaughter et al. (1981) have suggested that nucleosides and Hyp are transported by a common carrier. We (Plagemann & Wohlhueter, 1980) have argued against this interpretation on the basis of genetic evidence and differences of nucleoside and Hyp transport to inhibition by NBTI. Nucleoside transport in a variety of cells is strongly inhibited by NBTI (K_i about 1 nM), whereas Hyp transport, in at least some of the same cell types, has been shown to be little affected by concentrations of NBTI that close to saturate the high-affinity NBTI binding sites on the nucleoside carrier (Plagemann & Wohlhueter, 1980; Wohlhueter, Brown & Plagemann, 1983). Also, a mutant of mouse lymphoma S49 cells which is defective in nucleoside uptake (Cohen, Ullman & Martin, 1979) and lacks high-affinity NBTI binding sites (Cass et al., 1981) takes up Hyp unabated. Here we have further analyzed Hyp transport in a number of cell lines and show that two types of Hyp transporter can be distinguished on the basis of great differences in their sensitivities to inhibition by nucleosides and dipyridamole.

Abbreviations

Hyp, hypoxanthine; Urd, uridine; dThd, thymidine; NBTI, 6-(4-nitrobenzylthio)-9-β-D-ribofuranosyl purine; NBTP, 6-(4-nitrobenzylthio)purine; CHO cells, Chinese hamster ovary cells; CHL cells, Chinese hamster lung cells; EAT cells, Ehrlich ascites tumor cells; IC_{50}, concentration causing 50% inhibition.

Materials and Methods

Materials

[2-^{3}H]Hyp and [5-^{3}H]Urd were obtained from Moravek Biochemicals (Brea, Calif.) and diluted to desired specific radioactivities with unlabeled Hyp. Unlabeled Hyp and Urd were purchased from Sigma Chemical Co. (St. Louis, Mo.) and unlabeled NBTI from Calbiochem (San Diego, Calif.). Dipyridamole was a gift from Geigy Pharmaceuticals (Yongers, N.Y.). The synthesis of NBTP was carried out according to Johnson, Holman and Montgomery (1958). 6-Mercaptopurine · H_2O (Nutritional Biochemicals, Cleveland, Ohio, 1.12 g) plus K_2CO_3 (0.91 g, anhydrous) were dissolved in 5 ml of N,N-dimethylformamide and mixed slowly with *p*-nitrobenzylbromide (Aldrich, Milwaukee, Wis., 1.4 g dissolved in 2.5 ml N,N-dimethylformamide). The reaction was allowed to proceed 30 min at 40°C, and the product was crystallized from glacial acetic acid.

The crystalline product had a UV spectrum virtually identical to that of NBTI, but distinct from either starting reagent. In high-pressure liquid chromatography on a C18 reverse-phase column (Whatman-ODS-3, 2.5 × 250 mm, eluted isocratically with 55 vol methanol/45 vol 1 mM $(NH_4)_3PO_4$ + 1 mM NH_4Cl, pH 4.8) the product eluted as a homogeneous peak, somewhat later than NBTI, and much later than 6-mercaptopurine.

Cell Culture

Wild-type Novikoff rat hepatoma cells (N1S1-67) and an adenosine kinase and Hyp phosphoribosyltransferase-deficient variant thereof (1-22, Plagemann & Wohlhueter, 1983*a*) were propagated in suspension culture in Swim's medium 67 as described previously (Plagemann & Wohlhueter, 1982). Wild-type Chinese hamster ovary (CHO) cells, CHO-DAP12 cells, CHO-Aza^{r}ts, mouse L929-2 cells, mouse leukemia P388 and L1210, and rat hepatoma HTC cells were propagated in a similar manner in Eagle's Minimal Essential Medium for suspension culture supplemented with serum, nonessential amino acids, D-glucose, sera and Pluronic F68 as described previously (Plagemann & Wohlhueter, 1983*a*). CHO-DAP12 cells are deficient in adenine and Hyp phosphoribosyltransferase, whereas CHO-Aza^{r}ts is an azaguanine-resistant variant with normal levels of Hyp phosphoribosyltransferase (Harris & Whitmore, 1974). L929-2 is a varient of NCTC-929 cells which is deficient in adenine phosphoribosyltransferase (Puziss, Wohlhueter & Plagemann, 1983). A starter culture of HTC cells was kindly provided by Dr. Daryl Granner (University of Iowa). All cell lines were demonstrated to be free of mycoplasma contamination by uridine/uracil incorporation (Schneider, Stanbridge & Epstein, 1974). For experiments, cells were harvested by centrifugation from mid to late exponential phase cultures and suspended to 5 × 10^6 to 2 × 10^7 cells/ml of basal medium 42 (BM42B). EAT cells were propagated in Swiss mice (Levinson, 1972). A tumor-bearing mouse was kindly supplied by Dr. C. Levinson (University of Texas). Harvested cells (1 to 5 × 10^9 cells/mouse) were washed in balanced salt solution, suspended to 1 to 2 × 10^7 cells/ml of BM42B and used in transport experiments within 3 hr of harvest.

Hyp Transport

Hyp transport was measured in cell suspensions at 25°C as described previously (Plagemann & Wohlhueter, 1982). Time courses of transmembrane equilibration of radiolabeled Hyp were determined under zero-*trans* conditions by rapid kinetic techniques (15 time points per time course). The time intervals of sampling were progressively increased with increase in substrate concentration (*see* Plagemann & Wohlhueter, 1982) or inhibitor concentration (e.g., Fig. 1). Intracellular substrate concentration was expressed on the basis of intracellular water space determined by use of ^3H_2O. An integrated rate equation describing the time course of transmembrane equilibration of substrate in the zero-*trans* mode by a carrier with directional symmetry and equality of movement of empty and substrate-loaded carrier was fitted to the zero-*trans* data pooled for six to eight Hyp concentrations (Plagemann & Wohlhueter, 1982):

$$S_{2,t} = S_1\left[1 - \exp\left(-\frac{tV + (1 + S_1/K)S_{2,t}}{K + 2S_1 + S_1^2/K}\right)\right] \quad (1)$$

where $S_{2,t}$ = concentration of intracellular substrate at time t; S_1 = extracellular substrate concentration, K = Michaelis-Menten constant and V = maximum velocity. This equation was also fitted to time courses of Hyp and Urd equilibration across the membrane at single concentrations (320, 500 or 1000 μM), in which case K was fixed at 500 μM for Hyp (*see* Table 4) or 250 μM for Urd (Plagemann & Wohlhueter, 1984*b*). Initial zero-*trans* entry velocities (v_{12}^{tr}) were calculated for a given substrate concentration as the slopes of the equilibration curves described by Eq. (1) at $t = 0$: $v_{12}^{tr} = S_1V/(K + S_1)$.

In experiments designed to assess the effect of inhibitors on Hyp transport, dipyridamole, NBTI or NBTP were added to cell suspensions to the indicated concentrations at least 2 min prior to transport assay. Urd, at the indicated concentrations, was added simultaneously with labeled transport substrate.

The theoretical equations were fitted to data by a generalized least-squares regression program based on the algorithm of Dietrich and Rothmann (1975) and implemented on a Hewlett-Packard 9825 computer. Parameter values are reported ± SE of the estimate if not indicated otherwise.

Results and Discussion

Figure 1 illustrates representative time courses of transmembrane equilibration of 500 μM [^{3}H]Hyp by a Hyp phosphoribosyltransferase-deficient variant of Novikoff cells in the presence of the indicated concentrations of dipyridamole or Urd and illustrates the general method of measuring zero-*trans* influx in all experiments presented, both in these cells as well as in wild-type cells not deficient in Hyp phosphoribosyltransferase. In wild-type cells less than 10% of the intracellular radioactivity was

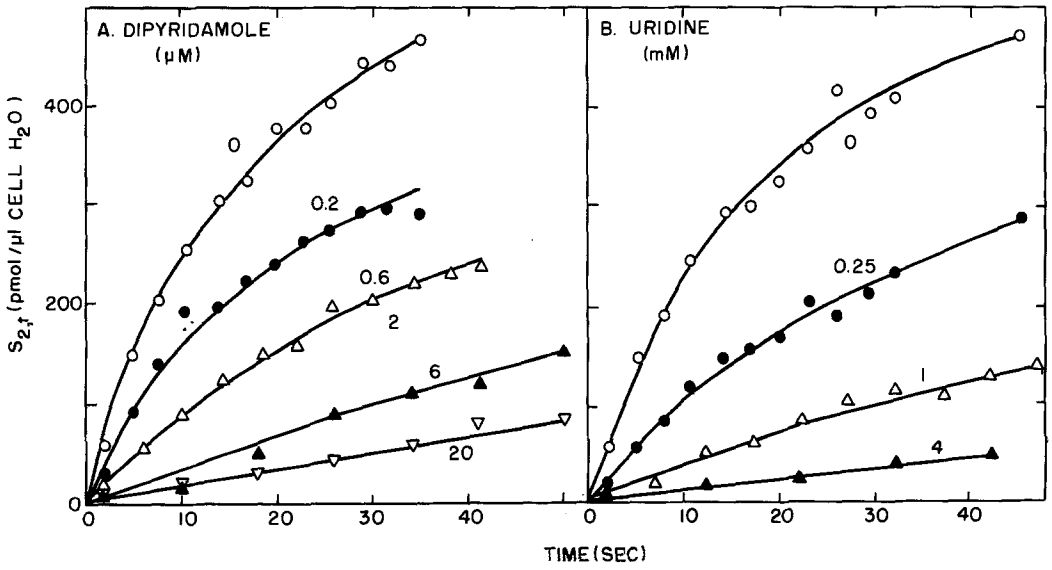

Fig. 1. Effect of dipyridamole and Urd on the zero-*trans* influx of Hyp in Hyp phosphoribosyltransferase-deficient Novikoff cells (1-22). *A*. Samples of a suspension of 1.1×10^7 cells/ml of BM42B were supplemented with the indicated concentrations of dipyridamole. Then the time course of transmembrane equilibration of 500 μM [^{3}H]Hyp (0.6 cpm/pmol) was measured at 25°C. *B*. Time courses of transmembrane equilibration of 500 μM [^{3}H]Hyp were determined in a suspension of 2.2×10^7 cells/ml in absence and presence of various concentrations of Urd. Urd was added simultaneously with radiolabeled Hyp. The initial velocities of zero-*trans* entry (v_{12}^{zt}) were computed by integrated rate analysis of the data as described under Materials and Methods. For the control cells, $v_{12}^{zt} = 47$ (in *A*) and 40 (in *B*) pmol/μl cell water · sec. The v_{12}^{zt} values for the inhibitor-treated cells are summarized in Fig. 2*A* and *B* as percent of control

associated with nucleotides after 1 min of incubation with 500 μM [^{3}H]Hyp (*data not shown; see* Plagemann & Wohlhueter, 1980). Thus, at this high Hyp concentration uptake time courses in wild-type cells, just as in Hyp phosphoribosyltransferase-deficient cells, reflected transmembrane equilibration of unmodified Hyp (*also see* Plagemann & Wohlhueter, 1982). This conclusion is further supported by comparable time courses of uptake of 500 μM Hyp by the Hyp phosphoribosyltransferase-deficient variant and wild-type Novikoff cells and similar effects of dipyridamole and Urd on Hyp uptake in these cells (*see later*). In the experiment illustrated in Fig. 1 and similar experiments with other cell lines and/or other inhibitors, initial zero-*trans* entry velocities (v_{12}^{zt}) were estimated as the zero-time slopes of the time courses of transmembrane equilibration (*see* Materials and Methods).

In Figure 2 the v_{12}^{zt} values for Hyp transport in a number of cell lines are plotted as a function of the concentrations of dipyridamole and Urd. The Hyp concentration of 500 μM was sufficient to achieve in most cell lines about 50% of maximal influx. In all instances inhibition of Hyp influx was dependent on inhibitor concentration, but two patterns of inhibition were observed. Hyp influx in wild-type Novikoff and CHO cells and their Hyp phosphoribosyltransferase-deficient variants, in HTC rat hepatoma cells and mouse EAT cells was strongly inhibited by dipyridamole (IC_{50} = 100 to 400 nM) and Urd (IC_{50} = 100 to 400 μM), whereas Hyp influx in mouse P388, L1210 and L929-2 cells was at least 100 times more resistant to dipyridamole ($IC_{50} > 30$ μM) and at least 10 times more resistant to Urd ($IC_{50} > 5$ mM).

Previous reports indicated that Hyp transport is highly resistant to inhibition by NBTI in CHO cells (Wohlhueter, Marz & Plagemann, 1978) and CHL cells (Slaughter et al., 1981). We have extended this finding to P388, L929-2 and EAT cells (Table 1). NBTI at a concentration of 1 μM inhibited Hyp influx only about 10 to 15%, whereas this concentration completely inhibits NBTI-sensitive nucleoside transport in these cells (*see later*). In addition, Hyp influx as well as Urd influx was little, if at all, affected by NBTP, the base counterpart of NBTI (Table 2).

Table 3 compares the sensitivities of Urd and Hyp transport in various cell lines to inhibition by NBTI, dipyridamole and each other. At least two groups of cells can be distinguished on the basis of these results. One group consists of P388, L1210 and L929-2 cells. In these cells Urd and Hyp transport are little affected by each other, which supports the view that Hyp and nucleosides are transported by distinct carriers. Nucleoside transport in these cells is highly sensitive to inhibition by NBTI and dipyridamole, whereas Hyp transport is highly

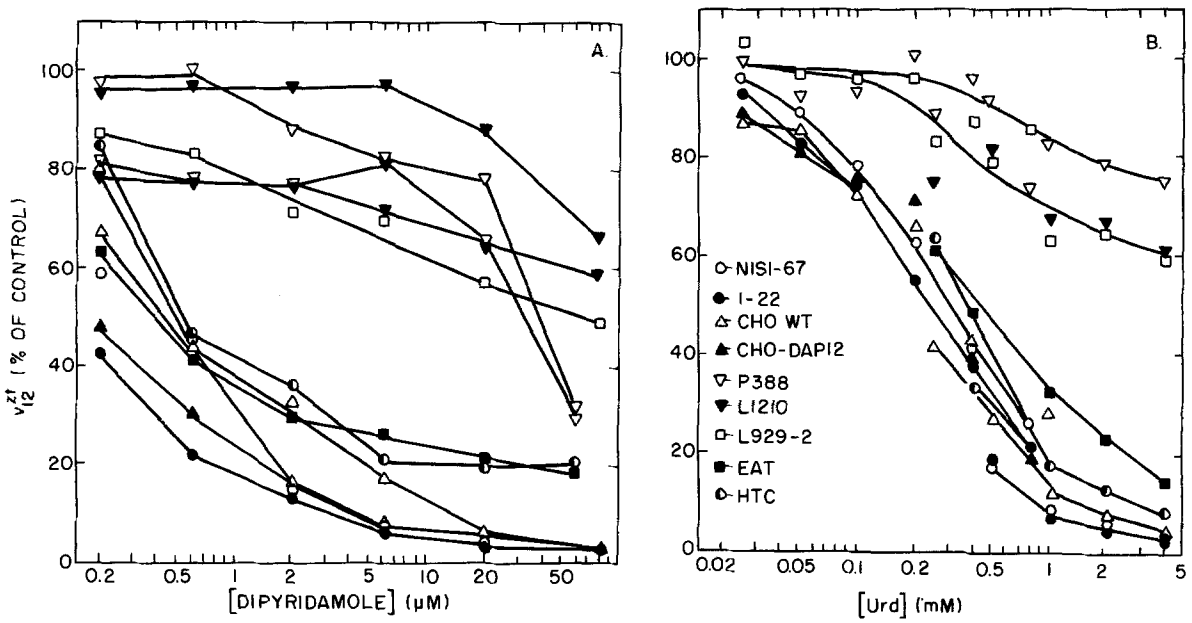

Fig. 2. Zero-*trans* Hyp influx in various cell lines as a function of concentration of dipyridamole (*A*) and Urd (*B*). Some of the values for 1-22 cells in *A* and *B* were calculated from the data in Fig. 1. All other experiments were conducted in the same manner as illustrated in Fig. 1. The v_{12}^{zt} values for untreated control cells ranged from about 10 pmol/μl cell water · sec for CHO and EAT cells to as high as 100 pmol/μl cell water · sec for P388, L1210 and Novikoff cells in the various experiments

Table 1. Effect of NBTI on Hyp transport in various types of cells[a]

Cells	v_{12}^{zt} (pmol/μl cell H_2O · sec)	
	Control	+1 μM NBTI
P388	35.1 ± 2.0	32.6 ± 1.2
L929-2	14.6 ± 0.8	12.6 ±× 0.4
EAT	5.7 ± 0.3	4.6 ± 0.3

[a] One portion of each cell suspension was supplemented with NBTI as indicated and the other portion remained untreated. Then the zero-*trans* influx of 500 μM [^{3}H]Hyp (0.6 cpm/pmol) was measured in duplicate as described in Materials and Methods.

Table 2. Effect of NBTP on the transport of Urd and Hyp in various cell lines[a]

Cell line	NBTP (μM)	v_{12}^{zt} (pmol/μl cell H_2O · sec)	
		Urd	Hyp
N1S1-67	0	6.5 ± 0.5	9.9 ± 0.7
	1	5.6 ± 0.2	7.6 ± 0.5
	5	6.7 ± 0.5	9.4 ± 0.6
CHO WT	0	4.5 ± 0.6	4.3 ± 0.4
	1	3.8 ± 0.5	4.4 ± 0.5
	5	3.1 ± 0.6	4.0 ± 0.3
L929-2	0	14.1 ± 0.9	25.7 ± 3.2
	1	10.7 ± 0.8	—
	5	8.5 ± 0.4	31.4 ± 4.8

[a] Samples of a suspension of each cell line were supplemented with NBTP as indicated and then the zero-*trans* influx of 320 μM Urd (1.5 cpm/pmol) and 320 μM Hyp (1.2 cpm/pmol) was determined as described in Materials and Methods.

resistant to both NBTI and dipyridamole. The high resistance of Hyp transport to inhibition by dipyridamole is unique to this group. Also characteristic of this group is the extent to which nucleoside transport is inhibited by nanomolar concentrations of NBTI (NBTI-sensitive transport, >85%). This inhibition is correlated with the binding of NBTI to high-affinity binding sites on the transporter ($K_d \lesssim 1$ nM; Wohlhueter et al., 1983; Plagemann & Wohlhueter, 1984*b*). The residual nucleoside transport in this group of cells is inhibited by NBTI only at concentrations >1 μM, as is the case for practically all nucleoside transport in Novikoff, HTC, Walker 256 carcinoma and CHL cells, in which cells high-affinity NBTI binding sites are absent (NBTI-resistant transport; Wohlhueter et al., 1978; Slaughter & Fenwick, 1981; Belt, 1983*a*,*b*). In CHO and EAT cells (Table 3) as well as in HeLa (Paterson et al., 1980; Dahlig-Harley, Eilam, Paterson & Cass, 1981) and MTC hamster cells (Heichal et al., 1978) NBTI-sensitive nucleoside transport comprises between 30 and 70% of total transport. We have postulated that the nucleoside transporter of mammalian cells may exist in sensitive and resistant

Table 3. Comparisons of effects of Urd and Hyp on the transport of each other and of NBTI and dipyridamole (DIP)[a]

Cell line	NBTI-sensitive Urd transport (% of total)	Inhibition – IC_{50}[b] (μM)					
		Urd Transport			Hyp Transport		
		NBTI	DIP	Hyp	NBTI	DIP	Urd
N1S1-67	<10	~10	1.0	~500	>5	0.1–0.4	100–500
HTC	<10	~10	1.0	~1000	ND[c]	~0.3	~200
CHO	60–70	0.003–0.006	0.2	⩾2000	>5	0.1–0.4	100–400
EAT	45–55	0.001	0.4	⩾2000	>5	0.4	~400
L929-2	>85	0.003–0.006	0.05	⩾2000	>5	>20	>5000
P388	>85	0.003–0.006	0.1	⩾2000	>5	>20	>5000
L1210	>85	0.003–0.006	0.3	⩾2000	ND	>20	>5000
CHL	<10	>1	ND	3300	>2	ND	4300

[a] Values for Hyp transport are estimated from results presented in Fig. 2*A* and *B*, and Table 1, except those for CHL cells which are from Slaughter and Fenwick (1981). Values for Urd transport are from Wohlhueter et al. (1978) and Plagemann and Wohlhueter (1984*a*,*b*).

[b] IC_{50} = concentration of inhibitor effecting a 50% decrease in v_{12}^{zt} at a substrate concentration of 500 μM.

[c] ND = not determined.

forms depending on its conformation in the membrane or interaction with additional membrane components to generate high-affinity NBTI binding sites (Plagemann & Wohlhueter, 1984*b*). The high-affinity binding of NBTI probably involves a hydrophobic association of its benzyl group with a site adjacent to, but distinct from, the nucleoside binding site of the carrier (Wohlhueter et al., 1983).

For the cell lines studied other than P388, L1210 and L929-2, the question of whether Urd and Hyp are transported by a common or distinct carriers is equivocal at present. Some results argue in favor of the operation of a common carrier, whereas other results are not readily compatible with this view. The most compelling argument for a common carrier in Novikoff, HTC, CHL, EAT and CHO cells is the finding that Urd and Hyp transport are inhibited by each other in a reciprocal manner approximately corresponding to the Michaelis-Menten constants of their transport. The Michaelis-Menten constants for Urd transport are about the same for all the cell lines in Table 3 we have investigated (150 to 300 μM, *see* Plagemann & Wohlhueter, 1984*b* and *unpublished data*). The Michaelis-Menten constants for Hyp transport, on the other hand, differed for these cell lines (Table 4), but in a manner unrelated to differences in the sensitivity of their Hyp transport system to inhibition by Urd and dipyridamole (Table 3). It was 400 to 600 μM for Novikoff, P388 and L929-2 cells, but >2000 μM for CHO and EAT cells as well as a line of Chinese hamster cells studied by Slaughter et al. (1981) (Table 4). The high K_m values must be considered rough estimates because the limited water solubility of Hyp precludes use of concentrations higher than 5 mM. The data in Table 3 show that the IC_{50} for the inhibition of Hyp transport by Urd in N1S1-67, HTC, CHO and EAT cells fell between 100 and 500 μM. The IC_{50} for inhibition of Urd transport by Hyp in N1S1-67 was about 500 μM. It was ⩾2000 μM for CHO and EAT cells, which is explained by the disparity between the K_m's for Urd and Hyp transport in these cells.

As pointed out already, Urd transport in the second group of cells (N1S1-67, HTC, CHO and EAT) differs in sensitivity to NBTI, but this difference is probably unrelated to carrier specificity and may reflect simply differences in the conformation of the carrier in the membrane. The differences in Michaelis-Menten constants for Urd and Hyp transport in different cell lines may speak against the idea that both are transported by a single carrier. On the other hand, when we directly compared Urd and Hyp influx at 1 mM in single batches of cells of each cell line, we found the ratio of v_{12}^{zt} for Hyp/v_{12}^{zt} for Urd to be about the same for most lines (Table 5), even though the individual rates for some cell lines differed between experiments. It was consistently about 2 for N1S1-67, HTC, P388 and L929-2 cells. The ratio was lower for CHO and EAT cells, but this was consistent with the higher K_m's for Hyp transport in these cells. The results indicate that there exists a constant relationship between the rates of Urd and Hyp transport. This relationship, however, held not only for the second group of cell types, but also for the first group of cell types with distinct Urd and Hyp transporters.

One serious problem with the single-carrier hypothesis is the finding that the binding of NBTI to high-affinity sites in CHO and EAT cells, as in

Table 4. Kinetic parameters for Hyp transport in various cell lines[a]

Cell line		Pretreatment	K (μM)	V (pmol/μl cell water · sec)
N1S1-67	1-9	None	508 ± 48	92 ± 12
	wild type	ATP-depleted	390 ± 25	68 ± 1.9
		ATP-depleted	352 ± 19	72 ± 1.8
CHO	wild type	ATP-depleted	2393 ± 221	41 ± 2
	Aza^{r}ts	None	2716 ± 327	37 ± 2
EAT		ATP-depleted	2976 ± 269	152 ± 5
L929-2		ATP-depleted	426 ± 41	38 ± 2
P388		ATP-depleted	445 ± 31	48 ± 1
CHL[b]		None	2000 ± 300	59 ± 2.7

[a] The experiments were conducted as described under Materials and Methods. Where indicated, the cells were depleted of ATP by preincubation in glucose-free BM42B containing 5 mM KCN and 5 mM iodoacetate (Plagemann, Marz & Erbe, 1976). The concentrations of radiolabeled Hyp were generally 60, 120, 240, 480, 960, 1820 and 3840 μM (500 to 900 cpm/μl, irrespective of concentration). Equation (1) was fitted to the pooled data and best-fitting parameters ± SE of estimate are listed, except in the case of the Hyp phosphoribosyltransferase-deficient variant of Novikoff cells (1-9) for which means ± SEM of 8 individual experiments are stated. Data for Novikoff and P388 cells are from Marz et al. (1979) and Plagemann and Wohlhueter (1982).

[b] Data of Slaughter et al. (1981); V was recalculated on the basis of 400 μg protein/μl cell water. Cells are Hyp phosphoribosyltransferase deficient.

Table 5. Direct comparison of Urd and Hyp influx in various cell lines[a]

Cell line		Experiment 1			Experiment 2		
		1 mM Urd v_{12}^{zt}	1 mM Hyp v_{12}^{zt}	v_{Hyp}/v_{Urd}	1 mM Urd v_{12}^{zt}	1 mM Hyp v_{12}^{zt}	v_{Hyp}/v_{Urd}
N1S1-67	wild type	12.9 ± 0.6	30.8 ± 1.2	2.4	11.7 ± 1.2	29.0 ± 1.0	2.5
	1-22	17.1 ± 0.7	28.0 ± 1.2	1.8	12.7 ± 0.4	30.1 ± 1.3	2.4
	1-14-7	24.9 ± 1.9	45.6 ± 3.4	1.8	18.3 ± 1.1	45.4 ± 1.6	2.5
HTC		5.8 ± 0.2	11.6 ± 0.7	2.0	ND[b]		
CHO	wild type	7.9 ± 0.4	7.2 ± 0.4	0.91	13.8 ± 0.9	9.6 ± 0.4	0.70
	Aza^{r}ts	12.8 ± 0.7	16.8 ± 1.1	1.3	6.6 ± 0.5	8.0 ± 0.5	1.2
EAT		18.5 ± 1.0	9.5 ± 0.4	0.51	7.9 ± 0.3	5.2 ± 0.4	0.66
P388		34.3 ± 1.6	85.1 ± 3.8	2.5	59.5 ± 2.8	103 ± 6.6	1.73
L929-2		22.2 ± 0.8	38.3 ± 2.3	1.7	30.8 ± 1.8	41.4 ± 2.0	1.4

[a] The zero-*trans* influx of 1 mM [^{3}H]Urd and 1 mM [^{3}H]Hyp was measured as described in Materials and Methods. The same cell population was analyzed for both Urd and Hyp influx.

[b] ND = not determined.

P388, L1210 and L929-2 cells, which results in inhibition of nucleoside transport, has no significant effect on Hyp transport. Slaughter et al. (1981) have suggested that the nucleoside binding site on the carrier is comprised of a ribose and a base recognition site and that the high-affinity binding of NBTI blocks only the ribose site and therefore inhibits nucleoside but not Hyp transport. It would follow that the inhibition of both Urd and Hyp transport by NBTI at concentrations above 1 μM must involve blockage of the base site also. But this explanation does not address the facts that adenine transport is not inhibited by nucleosides or Hyp in these cells, even though purine nucleosides are the nucleosides transported most efficiently by the carrier (Plagemann & Wohlhueter, 1980; 1983*b*), or that uracil transport is inhibited by nucleosides but not significantly by Hyp (Plagemann & Wohlhueter, 1980). Thus, even in cells where there seems to be a simple competition between nucleosides and Hyp for

transport, this simple reciprocity does not extend to other bases.

Slaughter et al. (1981) have applied their concept of NBTI binding to a ribose-recognition site to suggest that the transporter of the S49 variant deficient in nucleoside, but not Hyp, transport may be specifically defective in the ribose binding site. A more straightforward interpretation, however, is that S49 cells belong to the same group as P388, L1210 and L929-2 cells, which clearly possess distinct nucleoside and Hyp transporters. S49 cells are the only type of cultured cells which have been reported to possess a nucleoside transporter exclusively in the NBTI-sensitive form (Belt, 1983*b*; Plagemann & Wohlhueter, 1984*b*). Other properties of nucleoside and Hyp transport have not yet been defined for this line.

The failure of the base-analog of NBTI at a concentration of 1 or 5 μM to inhibit either base or nucleoside transport is pertinent to this issue. Assuming that failure to inhibit implies failure to bind suggests that the ribose moiety of NBTI is somehow crucial to its high-affinity binding. Just what role it plays is not clear; from the comparison of NBTI and inosine (Wohlhueter et al., 1983), it is apparent that the ribose moiety contributes little to the free energy of the NBTI/carrier complex.

Unambiguous answers to the question of specificity of transporters involved in nucleoside transport and nucleobase transport in various cells will require further genetic analysis and purification and reconstitution of the transporter(s). And, from the results presented here, we anticipate that the answers will be different for different types of cells.

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References

Belt, J.A. 1983*a*. Nitrobenzylthioinosine-insenstive uridine transport in human lymphoblastoid and murine leukemia cells. *Biochem. Biophys. Res. Commun.* **110**:417–423

Belt, J.A. 1983*b*. Heterogeneity of nucleoside transport in mammalian cells. Two types of transport activity in L1210 and other cultured neoplastic cells. *Mol. Pharmacol.* **24**:479–484

Cass, C.E., Kolassa, N., Uehara, Y., Dahlig-Harley, E., Paterson, A.R.P. 1981. Absence of binding sites for the transport inhibitor nitrobenzylthioinosine on nucleoside transport-deficient mouse lymphoma cells. *Biochim. Biophys. Acta* **649**:769–777

Cohen, A., Ullman, B., Martin, D.W., Jr. 1979. Characterization of a mutant mouse lymphoma cell with deficient transport of purine and pyrimidine nucleosides. *J. Biol. Chem.* **254**:112–118

Dahlig-Harley, E., Eilam, Y., Paterson, A.R.P., Cass, C.E. 1981. Binding of nitrobenzylthioinosine to high affinity sites on the nucleoside transport mechanism of HeLa cells. *Biochem. J.* **200**:295–305

Dietrich, O.W., Rothmann, O.S. 1975. Generalized linear least squares fitting. *Keyboard* (Hewlett Packard, Inc.) **7**:4–7

Harris, J.F., Whitmore, G.F. 1974. Chinese hamster cells exhibiting a temperature dependent alteration in purine transport. *J. Cell. Physiol.* **83**:43–52

Heichal, O., Bibi, O., Katz, J., Cabantchik, Z.I. 1978. Nucleoside transport in mammalian cell membranes. III. Kinetic and chemical modification studies of cytosine-arabinoside and uridine transport in hamster cells in culture. *J. Membrane Biol.* **39**:133–157

Johnson, T.P., Holman, L.B., Montgomery, J.A. 1958. Synthesis of potential anticancer agents. XVI. S-substituted derivatives of 6-mercaptopurine. *J. Am. Chem. Soc.* **80**:6265–6275

Levinson, C. 1972. Phosphate transport in Ehrlich ascites tumor cells and the effect of arsenate. *J. Cell. Physiol.* **79**:73–78

Marz, R., Wohlhueter, R.M., Plagemann, P.G.W. 1979. Purine and pyrimidine transport and phosphorylation and their interaction in overall uptake by cultured cells. *J. Biol. Chem.* **254**:2329–2338

Paterson, A.R.P., Lau, E.Y., Dahlig, E., Cass, C.E. 1980. A common basis for inhibition of nucleoside transport by dipyridamole and nitrobenzylthioinosine. *Mol. Pharmacol.* **18**:40–44

Plagemann, P.G.W., Marz, R., Erbe, J. 1976. Transport and countertransport of thymidine in ATP-depleted and thymidine kinase-deficient Novikoff rat hepatoma and mouse L cells: Evidence for a high K_m facilitated diffusion system with wide nucleoside specificity. *J. Cell. Physiol.* **89**:1–18

Plagemann, P.G.W., Wohlhueter, R.M. 1980. Permeation of nucleosides, nucleic acid bases and nucleotides in animal cells. *Curr. Top. Membr. Transp.* **14**:225–330

Plagemann, P.G.W., Wohlhueter, R.M. 1982. The hypoxanthine transporter of Novikoff rat hepatoma cells exhibits directional symmetry and equal mobility when empty or substrate-loaded. *Biochim. Biophys. Acta* **688**:505–514

Plagemann, P.G.W., Wohlhueter, R.M. 1983*a*. Adenosine metabolism in wild-type and enzyme-deficient variants of Chinese hamster ovary and Novikoff rat hepatoma cells. *J. Cell. Physiol.* **116**:236–246

Plagemann, P.G.W., Wohlhueter, R.M. 1983*b*. Adenosine and tubercidin binding and transport in Chinese hamster ovary and Novikoff rat hepatoma cells. *J. Cell. Physiol.* **116**:247–255

Plagemann, P.G.W., Wohlhueter, R.M. 1984*a*. Inhibition of the transport of adenosine, other nucleosides and hypoxanthine in Novikoff rat hepatoma cells by methylxanthines, papaverine, N^6-cyclohexyladenosine and N^6-phenylisopropyladenosine. *Biochem. Pharmacol.* **33**:1783–1788

Plagemann, P.G.W., Wohlhueter, R.M. 1984*b*. Nucleoside transport in cultured mammalian cells: Multiple forms with different sensitivity to inhibition by nitrobenzylthioinosine and hypoxanthine. *Biochim. Biophys. Acta* **773**:39–52

Puziss, M.D., Wohlhueter, R.M., Plagemann, P.G.W. 1983. Adenine transport and binding to cultured mammalian cells deficient in adenine phosphoribosyltransferase. *Mol. Cell. Biol.* **3**:82–90

Schneider, E.L., Stanbridge, E.F., Epstein, C.J. 1974. Incorporation of ^{3}H-uridine and ^{3}H-uracil into RNA. A simple technique for the detection of mycoplasma contamination of cultured cells. *Exp. Cell Res.* **84:**311–318

Slaughter, R.S., Barnes, E.M., Jr. 1979. Hypoxanthine transport in Chinese hamster lung fibroblasts: Kinetics and inhibition by nucleosides. *Arch. Biochem. Biophys.* **197:**349–355

Slaughter, R.S., Fenwick, R.G., Jr. 1981. Hypoxanthine and thymidine compete for transport in Chinese hamster fibroblasts. *Arch. Biochem. Biophys.* **211:**494–499

Wohlhueter, R.M., Brown, W.E., Plagemann, P.G.W. 1983. Kinetic and thermodynamic studies on nitrobenzylthioinosine binding to the nucleoside transporter of Chinese hamster ovary cells. *Biochim. Biophys. Acta* **731:**168–176

Wohlhueter, R.M., Marz, R., Plagemann, P.G.W. 1978. Properties of the thymidine transport system of Chinese hamster ovary cells as probed by nitrobenzylthioinosine. *J. Membrane Biol.* **42:**247–264

Wohlhueter, R.M., Marz, R., Plagemann, P.G.W. 1979. Thymidine transport in cultured mammalian cells. Kinetic analysis, temperature dependence and specificity of the transport system. *Biochim. Biophys. Acta* **553:**262–283

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