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# S49 mouse lymphoma cells are deficient in hypoxanthine transport

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**The rate of uptake of hypoxanthine in S49 cells was only about 2–5% of the rate of hypoxanthine transport observed in many other types of mammalian cells, and of the rate of uridine transport in this and other cell types. Part of the slow entry of hypoxanthine seems to be due to non-mediated permeation, but the remainder is saturable, strongly inhibited by uridine, nitrobenzylthioinosine and dipyridamole and not detectable in a nucleoside-transport-deficient mutant of S49 cells (AE1). The inhibition of hypoxanthine transport in S49 cells by nitrobenzylthioinosine resembles the inhibition of nucleoside transport in these and other mammalian cells, whereas it contrasts with the resistance of hypoxanthine transport to nitrobenzylthioinosine in all types of mammalian cells that have been investigated. We conclude that S49 cells lack the hypoxanthine transport system common to other types of cells and that hypoxanthine entry into these cells is mediated, although very inefficiently, by the nucleoside transporter. In contrast, adenine transport in S49 and AE1 cells was comparable to that in other types of cells.**

## Introduction

Mouse T lymphoma S49 cells have been shown to possess a nucleoside transport system with kinetic properties and substrate specificity comparable to that of other cultured cell lines [1,2]. Nucleoside transport in these cells, like in most mammalian cells, is inhibited by nanomolar concentrations of 6-((4-nitrobenzyl)thio)-9-β-D-ribofuranosylpurine (nitrobenzylthioinosine, NBTI), which is designated NBTI-sensitive transport, in distinction to a component of nucleoside transport, observed in most cell lines (10–100% of total), that is not inhibited by 1 μM NBTI and is designated NBTI-resistant transport [1,3,4]. Inhibition of nucleoside transport by NBTI correlates with the binding of NBTI to high-affinity binding sites ($K_d$ = 0.2–1 nM; about $1 \cdot 10^5$ binding sites/S49 cell; Refs. 1, 5, 6). Cohen et al. [7] have isolated a variant (AE1) of the mouse T-cell lymphoma S49 line which is defective in nucleoside transport. A defect in nucleoside transport in AE1 cells was indicated by a failure of the cells to take up a variety of radiolabeled nucleosides, by their increased resistance to the toxic effects of a variety of nucleoside analogues, and a lack of high-affinity NBTI binding sites [5,7]. It was also reported that the transport deficiency in AE1 cells is limited to nucleosides [7]. The sensitivity of AE1 cells to the toxic effects of various nucleobases was comparable to that of wild-type S49 cells, and their hypoxanthine uptake was only slightly lower than that observed for S49 cells, though overall uptake of hypoxanthine in both types of cells at 37°C in 30 min was less than 20% of that of adenosine, uridine or adenine. In contrast to the

Abbreviation: NBTI, 6-((4-nitrobenzyl)thio)-9-β-D-ribofuranosylpurine.

latter results, our present data show AE1 cells to take up hypoxanthine much more slowly than S49 cells. Even the wild-type S49 cells seem to lack a specific hypoxanthine transport system and hypoxanthine enters only very inefficiently via the nucleoside transport system. Thus, a defect in nucleoside transport in AE1 cells limits hypoxanthine uptake by these cells to non-mediated permeation.

## Experimental procedures

*Cell culture.* L1210 cells and Chinese hamster ovary (CHO) cells were propagated in suspension culture in Eagle's Minimal Essential Medium for suspension culture supplemented with non-essential amino acids, D-glucose, Pluronic F68 and 4% (v/v) fetal bovine serum, 4% (v/v) calf serum and 2% (v/v) newborn calf serum as described previously [8,11]. Cell suspensions were incubated in Erlenmeyer flasks of various sizes on a gyrotary shaker. S49 and AE1 cells were kindly supplied by Dr. B. Ullman and propagated in Eagle's Minimal Essential Medium supplemented with 5% (v/v) fetal bovine serum, non-essential amino acids and 10 mM additional D-glucose. Stock cultures were maintained in stationary suspension cultures in 75 and 150 $cm^2$ Corning tissue-culture flasks and subcultured at 2-day intervals by a 1:5 to 1:8 dilution with fresh growth medium. Two-liter Schott roller bottles were inoculated with 100–200 ml of suspension harvested from stock cultures and incubated on a roller machine at about 0.3 rpm. The cultures were expanded to 1.0–1.5 liters by daily addition of more growth medium. For experiments, cells were harvested from mid- to late-exponential-phase cultures and suspended to $(1-5)\cdot 10^7$ cells/ml of basal medium 42B (BM42B). Cells were enumerated in a Coulter counter.

*Transport and uptake of uridine and nucleobases.* Transport denotes solely the transfer of unmodified substrate across the cell membrane as mediated by a saturable, selective carrier. Uptake denotes the total intracellular accumulation of radioactivity from exogenous labeled substrate regardless of metabolic conversions. Uridine, adenine and hypoxanthine transport was measured in cell suspensions at 25°C as described previously [1,2,9,10]. Time courses of transmembrane equilibration of substrate were determined under zero-*trans* conditions by a rapid kinetic technique (15 time points per time course). Data were evaluated by fitting an integrated rate equation, based on the simple carrier model, with directional symmetry and equal mobility of empty and substrate-loaded carrier with the Michaelis-Menten constants fixed at 250 $\mu$M for uridine [1,2], at 500 $\mu$M for hypoxanthine [2,9], and 2 mM for adenine [2]. The slopes of the progress curves at $t = 0$ were taken as initial velocities ($v_{12}^{zt}$).

Longer time courses of substrate uptake were determined by supplementing samples of cell suspension with [5-$^3$H]uridine, [2-$^3$H]hypoxanthine, [8-$^{14}$C]hypoxanthine (Moravek Biochemicals, Brea, CA) or [8-$^{14}$C]adenine (Amersham Corp., Arlington Heights, IL) and inhibitors as indicated in appropriate experiments and sampling the suspensions manually during incubation at 25°C or other temperatures where indicated. The cells, from 0.5-ml samples of suspension, were collected by centrifugation through oil and analyzed for radioactivity [10]. In all experiments radioactivity values were corrected for substrate trapped in the extracellular space of cell pellets, which was estimated by the use of [$^{14}$C]inulin [10]. Concentrations of intracellular substrate equivalents were expressed on the basis of cell number or intracellular water space determined by the use of $^3H_2O$ [10].

*Other assays.* Uridine kinase and hypoxanthine phosphoribosyltransferase activities were measured in cell-free extracts with saturating concentrations of substrate as described previously [11,12]. Cell extracts were prepared from mid- to late-exponential-phase cultures comparable to those used in transport and uptake experiments.

## Results and Discussion

Fig. 1A illustrates the zero-*trans* influx of 500 $\mu$M uridine in S49 and AE1 cells. At this concentration, metabolic conversions of uridine are negligible during the time period of the transport assay [1]. The results confirm the absence of a functional transport system in AE1 cells [5,7]. The slow residual entry of uridine into AE1 cells can be attributed to non-mediated permeation; the ratio of the velocity of entry to the octanol parti-

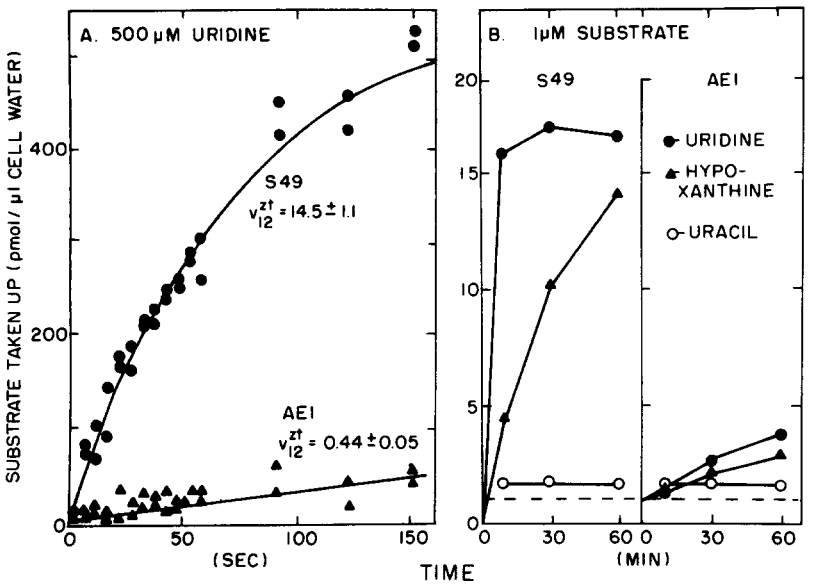


Fig. 1. Comparison of zero-*trans* influx of uridine (A) and of the uptake of uridine, hypoxanthine and uracil (B) in S49 and AE1 cells at 25°C. (A) The influx of 500 μM [$^3$H]uridine (1.4 cpm/pmol) was measured in duplicate by rapid kinetic techniques as described in Experimental procedures. Initial transport velocities ($v_{12}^{zt}$ in pmol/μl cell water per s) were estimated by integrated rate analysis. (B) Samples of suspensions of $5 \cdot 10^7$ S49 cells and $2.4 \cdot 10^7$ AE1 cells cells per ml of BM42B were supplemented with 1 μM [$^3$H]uridine (2000 cpm/pmol), 1 μM [$^3$H]hypoxanthine (480 cpm/pmol) or 1 μM [$^3$H]uracil (640 cmp/pmol). At various times of incubation, the cells from 0.5-ml samples of suspension were collected by centrifugation through oil and analyzed for radioactivity. All values are averages of duplicate samples. The broken lines indicate the intracellular substrate concentrations equal to those in the medium at zero time.

tion coefficient of uridine ($K_{oct} = 0.0149 \pm 0.00032$) was similar to the ratios observed for a number of other substances whose entry is limited to this route, such as L-glucose, cytosine and 8-azaguanine (see Ref. 2). Uridine influx in S49 cells was of the same order of magnitude as, but somewhat lower than, in another mouse leukemia cell line (L1210; $v_{12}^{zt} = 32.6 \pm$ pmol/μl cell water per s), which we assayed, for comparative purposes, in this and subsequent experiments. L1210 cells possess a nucleoside transport system with about the same kinetic properties and resistance of uridine transport to inhibition by hypoxanthine as that of S49 cells [1,4].

The nucleoside transport deficiency of AE1 cells explains their slow long-term incorporation of radiolabeled uridine (at 1 μM) into the nucleotide pool when compared to that of S49 cells (Fig. 1B), as well as their inability to grow in a medium (HAT) supplemented with methotrexate, thymidine and hypoxanthine (Table I). That the inability of AE1 cells to grow in HAT medium was

TABLE I

FAILURE OF THYMIDINE AND HYPOXANTHINE TO REVERSE GROWTH INHIBITION BY METHOTREXATE IN NUCLEOSIDE TRANSPORT MUTANT (AE1) OF S49 CELLS AND PREVENTION OF REVERSAL IN WILD-TYPE S49 CELLS BY TRANSPORT INHIBITORS

Suspensions of about $2 \cdot 10^5$ cells/ml were added to wells of 24-well tissue-culture plates (1 ml/well) and supplemented where indicated with 1 μM methotrexate (MTX), 100 μM hypoxanthine (Hyp), 15 μM thymidine (dThd), 1 μM NBTI and 5 μM dipyridamole (DIP). The plates were incubated at 37°C in a $CO_2$ incubator for 3 days and the cells in each well were enumerated with a Coulter counter. All values are averages from duplicate wells. The untreated wells of both types of cells (controls) contained $2.5 \cdot 10^6$ cells/well.

| Treatment | Cells/ml (% of control) | |
|---|---|---|
| | S49 | AE1 |
| + MTX | 10 | 6 |
| + MTX, dThd, Hyp | 65 | 10 |
| + MTX, dThd, Hyp, NBTI | 15 | 8 |
| + MTX, dThd, Hyp, DIP | 14 | 9 |
| + NBTI | 89 | 103 |
| + DIP | 90 | 71 |

related to their nucleoside transport defect is indicated by the finding that the growth of S49 cells in HAT medium was also almost completely inhibited by the two nucleoside transport inhibitors NBTI and dipyridamole (Table I). However, we found that the AE1 cells also took up hypoxanthine much more slowly than S49 cells (Fig.1B). Furthermore, we noticed that even wild-type S49 cells incorporated hypoxanthine only at about 10% of the rate observed in L1210 cells (data not shown) and much more slowly than uridine (Fig. 1B). In fact, the S49 cells had depleted the medium of [$^3$H]uridine by 10 min of incubation and uptake therefore had ceased. Fig. 1B also shows that S49 and AE1 cells failed to incorporate uracil, which is evidence for an absence of mycoplasma contamination [13].

Fig. 2 compares the time courses of uptake of various concentrations of hypoxanthine by the three cell lines (the medium was supplemented with a constant concentration of radiolabeled hypoxanthine plus increasing concentrations of unlabeled hypoxanthine). At all concentrations, hypoxanthine equilibrated across the membrane in L1210 cells within 30 s of incubation (Fig. 2A), which is as expected on the basis of the presence of an efficient hypoxanthine transport system in these cells [8]. The subsequent time course of uptake reflects the conversion of hypoxanthine to intracellular nucleotides (as previously demonstrated for other cell lines; Refs. 14 and 15) and the suppression of uptake of radioactivity by addition of increasing concentrations of unlabeled hypoxanthine is due to the saturation of hypoxanthine phosphoribosyltransferase ($K_m$ = 1–9 $\mu$M, see Ref. 15).

In S49 cells, in contrast, hypoxanthine had hardly attained an intracellular concentration equal to that in the medium by 4 min of incubation, even at the relatively low concentration of 10 $\mu$M (Fig. 2B). These results and the finding that the hypoxanthine phosphoribosyltransferase activity of S49 cells was comparable to that in L1210 cells (Table II) indicate that in S49 cells hypoxanthine influx was rate limiting in its conversion to intracellular nucleotides. This is also indicated by

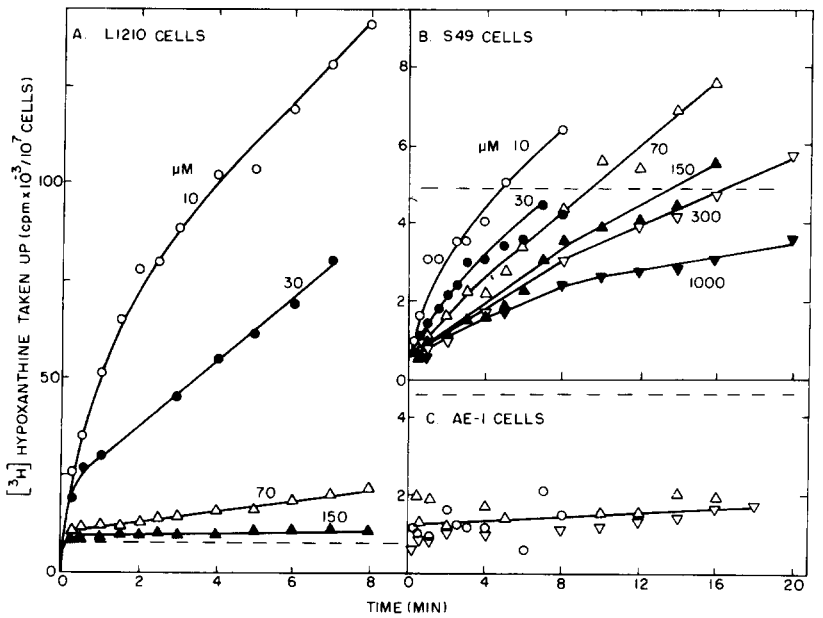


Fig. 2. Comparison of the uptake of [$^3$H]hypoxanthine by L1210, S49 and AE1 cells as a function of hypoxanthine concentration. Samples of suspensions of $2 \cdot 10^7$ L1210 cells, $1.9 \cdot 10^7$ S49 cells and $1.3 \cdot 10^7$ AE1 cells per ml of BM42B were supplemented with the indicated concentrations of [$^3$H]hypoxanthine (650 cpm/$\mu$l, irrespective of concentration). At various times of incubation at 25°C, the cells from 0.5-ml samples of suspension were collected by centrifugation and analyzed for radioactivity. All values are averages of duplicate samples. The broken lines indicate the intracellular concentration equal to that in the medium at zero time.

TABLE II

URIDINE (Urd) KINASE AND HYPOXANTHINE PHOSPHORIBOSYLTRANSFERASE (HPRT) ACTIVITIES OF VARIOUS CELL LINES

Results are expressed as pmol/min per mg protein.

| | Urd kinase | HPRT |
|---|---|---|
| S49 | 3.61 | 2.42 |
| AE1 | 2.10 | 5.08 |
| L1210 | 2.85 | 1.09 |
| P388 | 0.89 | 9.90 |

the observation that the saturation of uptake with increase in hypoxanthine concentration did not reflect the saturation of phosphoribosyltransferase as in L1210 cells. For the data in Fig. 2B, and those from another similar experiment, we calculated a $K_m$ for hypoxanthine uptake in S49 cells of between 500 and 700 $\mu$M. To what extent these values may represent the hypoxanthine transport $K_m$ in S49 cells is uncertain, because, on the one hand, the contribution of non-mediated permeation to total entry rate, which may be considerable (see later), was not considered in the calculation. On the other hand, at lower hypoxanthine concentrations, the saturation of phosphoribosylation may have affected, to some extent, the uptake time courses and thus the apparent uptake velocities.

In agreement with the data in Fig. 1, AE1 cells took up hypoxanthine even more slowly than S49 cells and there was no indication of saturation of uptake (Fig. 2C). Not even 30% of intracellular-extracellular equilibrium was attained in 20 min of incubation. The failure of hypoxanthine uptake by AE1 cells was not due to a lack of hypoxanthine phosphoribosyltransferase or *P*Rib-*PP*, since their hypoxanthine phosphoribosyltransferase activity (Table II) and rate of uptake of adenine (Fig. 3), respectively, were comparable to those in S49 cells. Indeed, chromatographic analysis of the acid-soluble pools of labeled AE1 and S49 cells indicated that most of the intracellular radioactivity was associated with nucleotides, which is consistent with a rate-limitation in uptake at the level of permeation rather than phosphoribosylation.

The finding that hypoxanthine uptake in S49 cells is saturable (Fig. 2B) indicated that at least part of the entry is mediated by a transport system. The finding that hypoxanthine uptake was highly temperature-dependent ($E_a$ = 21 kcal/mol; uptake rates were estimated from 7-point time courses at 5, 15, 20 and 35°C, not shown) is consistent with this view but does not prove it, since non-mediated substrate permeation is also highly temperature-dependent [2]. However, the $V_{max}$ for hypoxanthine uptake in S49 cells of about 0.5 pmol/$\mu$l cell per s, estimated from the data in Fig. 2B, is only about 1–2% of that observed for many other cultured cell lines [9]. These results could simply indicate that S49 cells possess a very inefficient hypoxanthine transport system. However, the finding that the nucleoside transport-deficient AE1 cells cannot transport hypoxanthine at all suggests an alternative interpretation of the results, namely that S49 cells lack the hypoxanthine transport system observed in other types of cells and that hypoxanthine entry into these

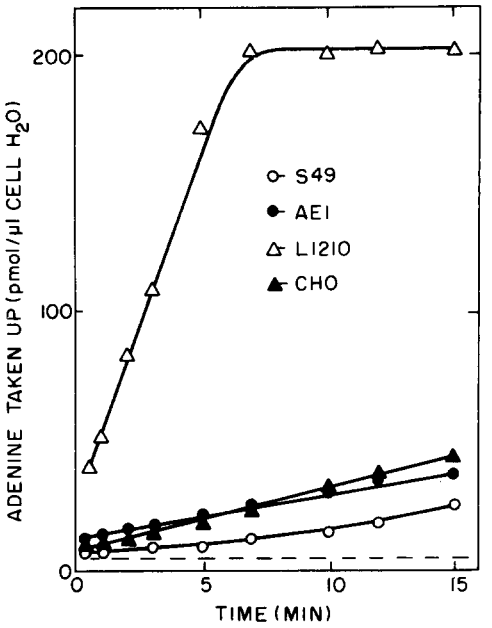


Fig. 3. Comparison of the uptake of adenine by S49, AE1, L1210 and CHO cells at 25°C. Samples of suspensions of $3.1 \cdot 10^7$ S49, $2.9 \cdot 10^7$ AE1, $2.7 \cdot 10^7$ L1210 and $6 \cdot 10^6$ CHO cells per ml of BM42B were supplemented with 4 $\mu$M [$^{14}$C]adenine (100 cpm/pmol). At various times of incubation, the cells from 0.5-ml of suspension were collected by centrifugation through oil and analyzed for radioactivity. All values are averages of duplicate samples. The broken line indicates the intracellular concentration of adenine equal to that in the medium at zero time.

cells is mediated by the nucleoside transporter, though very inefficiently (at about 1–2% of the rate of uridine translocation). This explains why a defect in nucleoside transport in AE1 cells abolishes hypoxanthine uptake altogether. Since the AE1 cells were isolated by single-step selection [6], it seems unlikely that they could possess a defect in both the nucleoside and hypoxanthine transporters, although the possibility is not ruled out that the nucleoside and hypoxanthine transporters might share a common component which is defective in AE1 cells.

Several lines of evidence argue against the latter possibility and support the view that hypoxanthine entry in S49 cells is mediated, though inefficiently, by the nucleoside carrier. First, uridine strongly inhibited the slow influx of hypoxanthine in S49 cells (Fig. 4B). One would expect hypoxanthine to similarly inhibit uridine transport if it is transported by nucleoside transporter, but this inhibition was difficult to demonstrate (Table III) probably because of marked differences in Michaelis-Menten constants for uridine and hypoxanthine transport. The same situation holds for some other cell lines in which uridine markedly inhibits hypoxanthine transport and both may be transported

TABLE III

EFFECT OF HYPOXANTHINE ON URIDINE TRANSPORT IN S49 CELLS

The zero-*trans* influx of 500 $\mu$M [$^3$H]uridine (1 cpm/pmol) was measured in samples of a suspension of about $1 \cdot 10^7$ S49 cells/ml by rapid kinetic techniques as described in Experimental procedures. The indicated concentrations of hypoxanthine were added simultaneously with the substrate. The initial transport velocities ($v_{12}^{zt}$) were estimated by integrated rate analysis.

| Hypoxanthine (mM) | Uridine influx (pmol/$\mu$l cell water per s) |
|---|---|
| 0 | 13.2 ± 0.7 |
| 0.25 | 13.9 ± 0.9 |
| 0.5 | 9.6 ± 0.7 |
| 1 | 9.4 ± 0.4 |

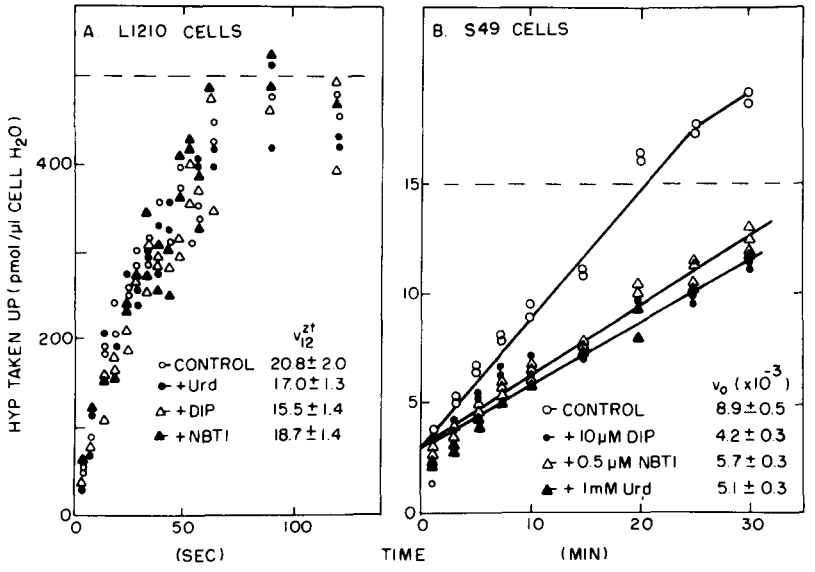


Fig. 4. Effects of uridine, dipyridamole and NBTI on zero-*trans* hypoxanthine influx in L1210 (A) and S49 (B) cells. In (A), samples of a suspension of $1.6 \cdot 10^7$ L1210 cells/ml of BM42B were supplemented with a 1.5 mM uridine, 20 $\mu$M dipyridamole (DIP) or 0.5 $\mu$M NBTI and then the influx of 500 $\mu$M [$^{14}$C]hypoxanthine (1 cpm/pmol) was measured by the rapid kinetic technique as described in Experimental procedures. Initial transport velocities ($v_{12}^{zt}$ in pmol/$\mu$l cell water per s) were estimated by integrated rate analysis. In (B), samples of a suspension of $2 \cdot 10^7$ S49 cells/ml of BM42B were supplemented with 15 $\mu$M [$^{14}$C]hypoxanthine (17 cpm/pmol) and where indicated at the same time with 1 mM uridine, 10 $\mu$M dipyridamole or 0.5 $\mu$M NBTI. At various times of incubation the cells from 0.5-ml samples of suspension were collected by centrifugation through oil and analyzed for radioactivity. Uptake velocities ($v_0$ in pmol/$\mu$l cell water per s) were estimated by linear regression of the uptake curves.

by a common carrier [1,9]. However, in most cell lines, including L1210, uridine has relatively little effect on hypoxanthine transport (see Fig. 4A) and, vice versa, hypoxanthine has little effect on uridine transport [1,9].

Second, hypoxanthine transport in S49 cells was strongly inhibited by NBTI and dipyridamole (Fig. 4B). The inhibition by dipyridamole is not conclusive, since hypoxanthine transport in some cell lines is also inhibited by dipyridamole, although in other cell lines, including L1210 cells, it is little affected (Ref. 9; see Fig. 4A). However, the inhibition of hypoxanthine uptake by NBTI in S49 cells is unique and resembles the inhibition of uridine transport in S49 cells and other types of cells investigated, whereas hypoxanthine transport in all other types of cell has been found to be resistant to NBTI inhibition.

Table IV compares the inhibition of uridine transport and hypoxanthine transport in S49 cells as a function of the NBTI concentration. 13% of total uridine transport was found to be NBTI-resistant, which contrasts with earlier results that indicated that nucleoside transport in the S49 cells is entirely in the NBTI-sensitive form [1,3,4]. We have no explanation for this difference and the molecular basis of NBTI-sensitive and resistant transport is not understood. However, the present experiments were conducted with a new batch of S49 cells received from Dr. Ullman and a change in the ratio of NBTI-resistant to NBTI-sensitive nucleoside transport has been observed in other cell lines [16]. In the case of hypoxanthine uptake, about 50% of the total was not inhibited by 50–1000 $\mu$M NBTI (Table IV). The nature of the NBTI-resistant uptake has not been elucidated, but on the basis of its magnitude and the octanol/water coefficient of hypoxanthine ($0.115 \pm 0.009$), it probably reflects non-mediated permeation. Nevertheless, the data in Table IV illustrate that the NBTI-sensitive components of uridine transport and hypoxanthine uptake in S49 cells exhibited similar sensitivity to NBTI inhibition ($IC_{50}$ between 1 and 5 nM).

In contrast to the defect in hypoxanthine transport, adenine transport in S49 cells was of the same order of magnitude as in L1210 cells (Table V) and other cell types [2] and about 100-times more rapid than hypoxanthine transport in these cells. Also, as in other cells, adenine transport in S49 cells was little, if at all, affected by NBTI, dipyridamole or uridine (Table V). These findings, combined with the observation that adenine transport was not reduced in AE1 cells (Table V), support our earlier suggestion that adenine is transported by a separate carrier. A rapid extracellular-intracellular equilibration of adenine, which was similar in S49 and AE1 cells and indi-

TABLE IV

INHIBITION OF URIDINE TRANSPORT AND HYPOXANTHINE UPTAKE BY NBTI IN S49 CELLS

About 5 min before assaying substrate uptake, samples of suspensions of $1.5 \cdot 10^7$ S49 cells/ml of BM42B were supplemented with NBTI to yield the indicated final concentrations. The zero-*trans* influx of 500 $\mu$M [$^3$H]uridine (1.2 cpm/pmol) was measured by rapid kinetic techniques as described under Experimental procedures. The uptake of 100 $\mu$M [$^{14}$C]hypoxanthine (4 cpm/pmol) was measured over a 30 min incubation period (see Fig. 4B) and the uptake velocities were estimated by linear regression of the utpake curves.

| Uridine (500 $\mu$M) | | | Hypoxanthine (100 $\mu$M) | | |
|---|---|---|---|---|---|
| NBTI (nM) | $v_{12}^{zt}$ (pmol/$\mu$l cell water per s) | % of control | NBTI (nM) | $V_0$ (pmol/$\mu$l cell water per s) | % of control |
| 0 | $4.12 \pm 0.27$ | 100 | 0 | $0.172 \pm 0.008$ | 100 |
| 0.3 | $3.17 \pm 0.21$ | 72 | 1 | $0.147 \pm 0.003$ | 85 |
| 1 | $2.69 \pm 0.15$ | 64 | 5 | $0.128 \pm 0.006$ | 74 |
| 3 | $1.05 \pm 0.08$ | 25 | 20 | $0.117 \pm 0.003$ | 68 |
| 10 | $0.60 \pm 0.05$ | 15 | 50 | $0.094 \pm 0.008$ | 54 |
| 50 | $0.52 \pm 0.03$ | 13 | 200 | $0.103 \pm 0.005$ | 59 |
| 150 | $0.53 \pm 0.12$ | 13 | 1000 | $0.089 \pm 0.004$ | 52 |
| 500 | $0.55 \pm 0.04$ | 13 | | | |

TABLE V

ADENINE TRANSPORT VELOCITIES IN S49, AE1 AND L1210 CELLS AND LACK OF INHIBITION BY NBTI, DIPYRIDAMOLE AND URIDINE

Samples of suspensions of $1.4 \cdot 10^7$ S49 cells, $1.6 \cdot 10^7$ AE1 cells and $1.7 \cdot 10^7$ L1210 cells per ml of BM42B were supplemented, where indicated, with 20 $\mu$M dipyridamole, 1 $\mu$M NBTI or 1.5 mM uridine and then the zero-*trans* influx of 500 $\mu$M [$^{14}$C]adenine (650 cpm/nmol) was measured by the rapid kinetic technique described in Experimental procedures. The initial transport velocities ($v_{12}^{i}$) were estimated by integrated rate analysis.

| Treatment | Adenine influx (pmol/$\mu$l cell water per s) | | |
|---|---|---|---|
| | S49 cells | AE1 cells | L1210 cells |
| None | 16.6 ± 3.4 | 18.4 ± 3.6 | 41 ± 18 |
| Dipyridamole | 14.5 ± 3.1 | 15.7 ± 2.6 | 24 ± 4.4 |
| NBTI | 13.9 ± 2.1 | 17.2 ± 2.7 | 34 ± 8.5 |
| Urd | 19.5 ± 3.8 | 14.4 ± 2.1 | 35 ± 7.4 |

cative of a functional transport system, was also apparent from the uptake experiments shown in Fig. 3. However, in three independent uptake experiments, we observed that, within 30 s of incubation of AE1 cells with 4 $\mu$M adenine, the latter attained an apparent intracellular concentration 2–3-times that in the medium or observed in wild-type S49 cells (see Fig. 3), whereas no similar concentrative adenine accumulation was observed in a zero-*trans* influx experiment with 500 $\mu$M adenine (Table V). These results suggest that AE1 cells, but not wild-type S49 cells, may possess saturable adenine-binding components similar to those observed for L929 cells ($K_d$ about 18 $\mu$M; Ref. 17). The rates of long-term uptake of adenine by S49 and AE1 cells, though about the same for both cell types, were much lower than that observed for L1210 cells, but similar to that of CHO cells (Fig. 3). We have not ascertained whether these differences are related to differences in adenine phosphoribosyltransferase activities or available *P*-Rib-*PP* concentrations.

The reason for the lack of a hypoxanthine transporter in S49 cells is unknown. It might be related to the T-lymphocyte origin of these cells. We are not aware of any studies on hypoxanthine transport in mouse T-lymphocytes. On the other hand, a loss of hypoxanthine transporter could have occurred as a result of transformation or continuous cultivation in vitro.

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