Adenine Transport and Binding in Cultured Mammalian Cells Deficient in Adenine Phosphoribosyltransferase

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Rapid kinetic techniques were employed to measure the transport of adenine in adenine phosphoribosyltransferase-deficient L929 and Chinese hamster ovary (CHO) cells in zero-*trans* entry and exit and equilibrium exchange procedures. The kinetic parameters of transport were computed by fitting appropriate integrated rate equations to time courses of transmembrane equilibration of radiolabeled adenine. Adenine transport conformed to the simple carrier model with directional symmetry and equal mobility of loaded and empty carrier. The Michaelis-Menten constants and maximum velocities for various strains of L929 cells fell between 2.3 and 3.5 mM and 90 and 150 pmol/ μ l of cell water per s, respectively, values similar to those previously reported for CHO and Novikoff hepatoma cells. The corresponding values for hypoxanthine transport in L929 cells were 413 μ M and 16 pmol/ μ l of cell water per s. Adenine transport velocities were directly proportional to adenine concentrations between 0.03 and 50 μ M in both CHO and Novikoff cells. The results indicate that adenine is transported in these cells by a single, low-affinity, high-capacity transporter. Adenine transport was inhibited by hypoxanthine in some cell strains, but not in others. Adenine also rapidly bound to L929 cells in a saturable manner ($K_D = 18 \mu$ M), presumably to the cell surface (about 3×10^7 sites per cell).

In a previous study (6) we applied rapid kinetic techniques to measure adenine transport in Novikoff rat hepatoma and Chinese hamster ovary (CHO) cells in which adenine phosphoribosylation was prevented by prior depletion of the cells of ATP and phosphoribosylpyrophosphate or by a genetic deficiency in adenine phosphoribosyltransferase (APRT; EC 2.4.2.7). (Transport denotes solely the transfer of unmodified exogenous substrate across the cell membrane as mediated by a saturable, selective carrier.) Transport is very rapid; the half-time for transmembrane equilibration in the zero-*trans* mode in the first-order substrate range at 25°C is about 10 s. (As defined by Eilam and Stein [4], zero-*trans* designates the transport of a substrate from one side of the membrane to the other side, where its concentration is zero. Equilibrium exchange is the unidirectional flux of radiolabeled substrate from one side of the membrane to the other side, where substrate is held at equal concentration. Arbitrarily, we designate the outside and inside faces of the membrane as 1 and 2, respectively.) The Michaelis-Menten constant for adenine transport of between 2 and 4 mM was found to be much higher than values reported earlier for the uptake of adenine by cells with functional APRT activity (0.2 to 100 μ M; for summary see reference 11) where uptake

denotes the transfer of radioactivity from exogenous labeled substrate to intracellular space or components regardless of metabolic conversions. We suggested that the latter values probably reflected the saturation of the intracellular phosphoribosylation of adenine rather than of its transport into the cell, just as has been demonstrated for nucleosides and hypoxanthine (11).

This view, however, has been challenged. Templeton and Chilson (17) recently reported a Michaelis-Menten constant for adenine uptake in rabbit erythrocytes of 0.5 μ M and suggested that we might have missed detecting the high-affinity transport system because the lowest concentration of adenine we tested was 0.5 μ M. Suresh and co-workers (14, 15) reported Michaelis-Menten constants of 0.07 and 17 μ M for adenine uptake in wild-type L1210 cells, which were interpreted to reflect the operation of two high-affinity adenine transporters. It seems more likely, however, that in both of these studies also saturation of intracellular adenine phosphoribosylation rather than of transport was measured, since uptake was linear for 1 to 2 min, and the Michaelis-Menten constant for adenine uptake by an APRT-deficient variant of L1210 cells was 2.8 mM, a value comparable to those for Novikoff and CHO cells (6, 11).

Nevertheless, in the present study we have

reexamined whether such high-affinity adenine transport systems are present in CHO and L cells deficient in APRT. None was detected. Incidentally, however, we found rapid saturable binding of adenine to L cells, presumably to cell surface components, which complicates transport measurements in these and possibly other types of cells. We have also further assessed the substrate specificity of the system transporting adenine.

MATERIALS AND METHODS

Cells and cell culture. Ltk⁻ cells were obtained from W. Munyon in 1974; L241 cells (a strain of L2), a subline of L929 which has been selected for sensitivity to mouse hepatitis virus (5), were obtained from Martin V. Haspel in 1979; L929 cells (NCTC 929, passage 556) were obtained from the American Type Culture Collection; and diaminopurine-resistant CHO cells (DAP12 [16]) were obtained from Milton W. Taylor. Ltk⁻ and L241 cells, like CHO DAP12 cells, are deficient in APRT, and L929-2 is an APRT-deficient clone of L929 cells we have isolated (Puziss and Plagemann, unpublished data). These cell strains were propagated in suspension culture as previously described for Novikoff rat hepatoma cells (10, 12) in modified Eagle medium for spinner culture supplemented with nonessential amino acids, D-glucose to a final concentration of 16 mM, 0.53 mg of Pluronic F-68 (10 per ml, 4% (vol/vol) horse serum, 4% (vol/vol) calf serum, and 2% (vol/vol) newborn calf serum. Novikoff cells were propagated in Swim's medium 67 (12). All cell lines were ascertained to be free of mycoplasma by the uridine and uracil incorporation (13) and cultural methods.

Measurement of adenine transport. Cells were collected by centrifugation from exponential phase cultures and suspended in basal medium 42B (BM42B [8]) to about 10^7 cells per ml.

Adenine entry and exit and equilibrium exchange were measured by a rapid mixing and sampling method described in detail previously (19). For zero-trans entry measurements, samples of cell suspension and a solution of radiolabeled substrate were mixed at short intervals in a ratio of 7.3:1 with a dual syringe apparatus, and substrate accumulation was terminated by collecting the cells by centrifugation through oil. The kinetic parameters of adenine transport were estimated by fitting the following integrated-rate equation (which pertains to a simple carrier with directional symmetry and equal mobility of substrate-loaded and empty carrier [11]) to time courses of substrate accumulation to transmembrane equilibrium pooled for seven substrate concentrations:

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

where $S_{2,t}$ is the concentration of substrate at time t ($S_{2,0} = 0$), S_1 is the exogenous concentration of substrate (and taken as a constant), V is the maximum velocity, and K is the Michaelis-Menten constant. Initial velocities of zero-trans entry (v_{12}^z) were calculated for a given substrate concentration (S_1) as the

slope of the best fitting curve at $t = 0$, $v_{12}^z = S_1 V / (K + S_1)$.

For estimating v_{12}^z for adenine uptake at a single concentration $\ll K$, we fitted the following exponential equation to time courses of adenine uptake at a single concentration:

$$S_{2,t} = (S_{2,\infty} - S_{2,0})[1 - \exp(-kt)] + S_{2,0} \quad (2)$$

where k is the first-order rate constant, $v_{12}^z = kS_1$ ($S_1 = S_{2,\infty} - S_{2,0}$), and $S_{2,0}$ is an estimate of the amount of substrate rapidly bound to cells, if such occurs. When no significant binding was detectable, $S_{2,0}$ was fixed at zero and $S_{2,\infty} = S_1$.

Time courses of inward equilibrium exchange were determined in the same manner, except that the cells were preincubated at 37°C for 15 min with unlabeled substrate at the same concentration as radiolabeled substrate used in the uptake measurement.

For adenine exit and outward equilibrium exchange measurements suspensions of about 10^8 cells per ml were equilibrated with specified concentrations of radiolabeled adenine at 37°C for 15 min. After equilibration at 25°C, samples of the suspension of preloaded cells were mixed with the dual syringe apparatus at a ratio of 1:7.3 with BM42B (exit) or BM42B containing the same concentration of unlabeled substrate as used for preloading (outward equilibrium exchange).

The time course of isotopic exchange at equilibrium is given by (11):

$$N_{2,t} = N_{2,\infty} \left[1 - \exp \left(- \frac{V^{ee} t}{K^{ee} + S} \right) \right] \quad (3)$$

for inward exchange and:

$$N_{2,t} = (N_{2,0} - N_{2,\infty}) \left[\exp \left(- \frac{V^{ee} t}{K^{ee} + S} \right) \right] + N_{2,\infty} + N_{2,x} \quad (4)$$

for outward exchange, where $N_{2,t}$ is the intracellular concentration of radioactivity at time t ; S is the substrate concentration which is equal on both sides of the membrane, and K^{ee} and V^{ee} are the apparent Michaelis-Menten constant and maximum velocity for equilibrium exchange, respectively. For inward exchange, $N_{2,\infty} = N_1$, the concentration of radioactivity per equivalent volume of medium. For outward exchange, $N_{2,\infty} = N_{2,0}/8.3$, and not 0, because of the carryover of radioactivity from the preloading mixture into the extracellular compartment which our procedure entails. In equation 4, $N_{2,x}$ is equal to nonexchangeable radioactivity present intracellularly after preloading, which, in the case of adenine uptake, might reflect amounts of bound adenine. To facilitate comparison with net flux data, N_2 has been expressed as chemical concentration of radiolabeled substrate at its initial specific radioactivity. Initial equilibrium exchange (v^{ee}) velocities were calculated for a given substrate concentration as the slopes of the curves described by equations 3 and 4 at $t = 0$: $v^{ee} = S V^{ee} / (K^{ee} + S)$. Equations 1 through 4 were fitted by a nonlinear least-squares regression program based on the algorithm of Dietrich and Rothmann (3). We have so far not been able to apply integrated rate analysis satisfactorily to time courses of zero-trans exit of adenine. One prob-

FIG. 1. Zero-trans uptake of 0.03 to 50 μM adenine by CHO DAP12 cells at 24°C. The uptake of 0.03, 0.13, 0.43, 2, 8, 20, and 50 μM [^3H]adenine (789 cpm/ μl , irrespective of concentration) was measured by the rapid kinetic technique described in the text. Radioactivity per cell pellet was corrected for adenine trapped in extracellular space (3.4 μl per pellet) and converted to picomoles per microliter of cell water on the basis of an intracellular water space of 12 μl per cell pellet. Equation 2 was fitted to each uptake time course with $S_{2,0}$ fixed at 0. Two representative initial time courses are shown in A and B. $S_{2,t}$ is in picomoles per microliter of cell water. v_{12}^{zt} (in picomoles per microliter of cell water per s) was calculated as the slope of the best-fitting curve at $t = 0$. In C the computed v_{12}^{zt} values (●) as well as those from a second duplicate experiment (▲) are plotted as a function of the extracellular adenine concentration (S_1).

lem is the presence of noneffluxable radioactivity in preloaded cells, referred to above, which introduces an additional parameter to be fitted. Initial velocities of zero-trans exit (v_{21}^{zt}) were, therefore, estimated graphically.

Countertransport (infinite-trans) was measured as follows: samples of suspension of about 10^7 cells per ml of basal medium were preincubated with various nucleosides or nucleobases at a concentration of 5 mM at 37°C for 15 min. Then the cells were collected by centrifugation and rapidly suspended to the same density in basal medium containing 10 μM [^3H]adenine or hypoxanthine. At various times of incubation at 25°C, the cells from 0.5 ml of suspension were collected by centrifugation through oil and analyzed for radioactivity. Where indicated, cells were depleted of ATP (and phosphoribosylpyrophosphate) by preincubation in glucose-free BM42B containing 5 mM KCN and 5 mM iodoacetate as described previously (9).

Materials. [$8\text{-}^{14}\text{C}$]adenine and [$8\text{-}^3\text{H}$]adenine were purchased from Amersham Corp. and diluted with unlabeled adenine (Sigma Chemical Co.) to the desired specific radioactivity. The radiolabeled adenines were

>98% pure as assessed by high-pressure liquid chromatography.

RESULTS AND DISCUSSION

In our search for the existence of a high-affinity adenine transporter we measured the initial velocities of transport of adenine by the APRT-deficient CHO DAP12 cells over a range of 0.03 to 50 μM by fitting an exponential rate equation (equation 2) to the initial time courses of adenine uptake (representative time courses with 0.13 and 20 μM adenine are shown in Fig. 1A and B, respectively). In two experiments, the initial transport velocities were directly proportional to the adenine concentration (Fig. 1C). The mean first-order rate constant ($k = 0.0328 \text{ s}^{-1}$) was similar to that predicted from the kinetic parameters for adenine transport in these cells determined previously ($V/K = 0.0185$ to 0.0219 s^{-1} [6, 11]). We conclude, therefore, that

FIG. 2. Zero-trans uptake of 0.03 to 50 μM adenine by L929-2 cells at 24°C. The experiment was conducted as described in the legend to Fig. 1, except that $S_{2,0}$ was treated as a parameter to be fitted in the integrated rate analysis of the uptake time courses (see text). The radioactivity concentration was 256 cpm/ μl , irrespective of the adenine concentration, and the cell water and extracellular water spaces per cell pellet were 20.2 and 4.7 μl , respectively. The broken lines indicate the extracellular concentration of adenine at 0 time (picomoles per microliter of culture fluid).

these cells possess only a single low-affinity, high-capacity adenine transport system.

When we conducted similar experiments with various strains of L cells that lack APRT we found, unexpectedly, that some adenine became very rapidly associated with the cells (within 2 s), which could not be accounted for by transport into the cells, and that overall uptake was apparently concentrative (Fig. 2). Since the rapid association of adenine with cells was only slightly lower at 4°C than at 25°C (data not shown) we suggest that it reflects rapid binding of adenine to cell surface components. Furthermore, the decrease in the relative amount of adenine that became associated with the cells during the first 2 s with the increase in adenine concentration suggested that this binding was saturable. We have estimated the amounts bound and the initial transport velocities by fitting equation 2 to the uptake time courses whereby $S_{2,0}$ was treated as a parameter to be fitted (Fig. 2). The best-fitting $S_{2,0}$ (y -intercept) values, expressed as concentration of bound

adenine, conformed well to a one-component binding isotherm (Fig. 3A) with a K_D of 15.2 μM and 3.3×10^7 binding sites per cell. Scatchard plots of the data by the method of Weder et al. (18) showed a single straight line (Fig. 3B). In a second approach we measured the amount of adenine taken up by the cells at steady state (30 min of incubation at 4°C) as a function of adenine concentration and found these values to conform to a two-component binding system (Fig. 3C and D). The parameters for the high-affinity system were similar to those estimated from 0-time uptake (Fig. 3A). Similar results were obtained in three independent but comparable experiments (Table 1). The low-affinity component, which shows no detectable saturation below 40 μM , probably reflected the amounts of adenine accumulated within the cells via the transport system.

The initial velocities of adenine transport (corrected for surface binding) by L cells obtained in three independent experiments were directly proportional to adenine concentration between 0.03 and 50 μM (Fig. 4) just as in the case of the CHO DAP12 cells. And, as in the case of the CHO DAP12 cells, the mean first-order rate constant was that predicted from the kinetic parameters of the low-affinity transporter (see below).

Since surface binding of adenine by L cells is saturated at relatively low concentrations of adenine, transport can be measured at higher concentrations of adenine uncomplicated by binding. First we compared zero-trans influx and efflux and zero-trans and equilibrium exchange fluxes of adenine and found all fluxes to be about the same (Fig. 5). The time courses of change in intracellular concentration of radiolabeled adenine in the zero-trans and equilibrium exchange protocol ($S_{2,t}$ and $N_{2,t}$, respectively) in Fig. 5 differed, but this difference is entirely predictable from the appropriate integrated rate equations based on the simple carrier model. (For further documentation see reference 12; at concentrations of substrate $\ll K$, the time courses of zero-trans entry and inward equilibrium exchange are identical.) Equality of zero-trans influx and efflux proves directional symmetry of the adenine transporter, and equality of zero-trans and equilibrium exchange fluxes proves equal mobility of empty and adenine-loaded carrier (11). These properties had been postulated previously (6) on the basis of integrated rate analysis of zero-trans influx in Novikoff and CHO cells, but had not been proven directly. The results validate our approach of estimating the kinetic parameters for adenine transport by integrated rate analysis of zero-trans influx alone.

Figure 6 illustrates representative time

FIG. 3. Binding of adenine to L cells. (A) The details of the experiment are described in the legend to Fig. 2. The computed amounts of adenine associated with the cells at 0 time ($S_{2,0}$) were expressed as concentration of bound ligand (L_b), and the following one-component binding isotherm (18) was fitted to the combined data: $L_b = NL_f + (K_D L_f)$, where L_f is concentration of free ligand, K_D is the dissociation constant, and N is the concentration of binding sites. (B) Scatchard plot of the data by the method of Weder et al. (18), with K_D and N fixed at the values computed in A. (C) Samples of a suspension of L241 cells were supplemented with 0.018, 0.056, 0.096, 0.26, 0.82, 1.63, 8, 26, 200, 500, and 1,000 μM [^3H]adenine (228 cpm/ μl , irrespective of concentration) at a final cell density of 6×10^7 cells per ml. After 30 min of incubation at 4°C the cells from duplicate 0.5-ml samples of the suspension were collected by centrifugation through oil and analyzed for radioactivity. Radioactivity per cell pellet was corrected for adenine trapped in extracellular space (12.8 μl per pellet) for an estimate of the concentration of bound ligand. A sample of the supernatant fluid was analyzed for radioactivity to estimate the concentration of free ligand. A two-component binding isotherm (18):

$$L_b = \frac{N_1 L_f}{K_{D,1} + L_f} + \frac{N_2 L_f}{K_{D,2} + L_f}$$

was fitted to the data. The broken lines indicate the contributions of each of the two components. (D) Scatchard plot of the data by the method of Weder et al. (18) with K_D and N fixed at the values computed in C. The reported parameters are for the high-affinity component.

courses of zero-trans uptake of adenine by L241 cells at concentrations well above the binding K_D . Integrated rate analysis of the combined data for uptake at seven concentrations of adenine ranging from 80 to 5,120 μM yielded K and

V values of 3049 μM and 91 pmol/ μl of cell water per s, respectively. Similar values were obtained in comparable experiments with L929-2 and Ltk⁻ cells (Table 2). These values are quite comparable to those previously obtained for

TABLE 1. Equilibrium binding of [^3H]adenine by L241 cells at 4°C ^a

Expt	N (μM)	K_D (μM)	Binding sites/cell	Correlation coefficient ($r_{y,g}$)
1	3.49 ± 0.13	17.2 ± 1.3	4.26×10^7	0.999
2	2.71 ± 0.043	16.7 ± 0.96	3.05×10^7	0.999
3	3.29 ± 0.044	19.7 ± 0.54	3.70×10^7	1.000
Mean	3.16 ± 0.23	17.9 ± 0.93	$3.67 \times 10^7 \pm 0.35 \times 10^7$	

^a Experiment 1 is described in detail in the legend to Fig. 3C. All values are expressed $\pm$ the standard errors of the estimates, except for the means $\pm$ standard errors of the means.

FIG. 4. Initial velocities of adenine transport in L cells as a function of extracellular adenine concentration between 0.03 and 50 μM . The details of one of the experiments with L929-2 cells (●) are described in the legend to Fig. 2. The duplicate experiment with L929-2 cells and the experiment with L241 cells were conducted and analyzed in the same manner.

Novikoff and CHO cells and indicate that all of these cell lines possess a single low-affinity adenine transport system with similar properties. The K and V values for hypoxanthine transport in ATP-depleted L929 cells determined in the same manner as described for adenine were $413 \pm 43 \mu\text{M}$ and $16.2 \pm 0.5 \text{ pmol}/\mu\text{l}$ of cell water per s, respectively. These values are also similar to those previously reported for another strain of L cells and other mammalian cell lines (6).

Figure 7A shows that preloading of L241 cells with 5 mM adenine, hypoxanthine, or uracil caused a transient intracellular, concentrative accumulation of 10 μM [^3H]adenine (countertransport), whereas preloading with thymidine had no effect. These results are similar to those reported by Cybulski et al. (2) for L1210 mouse leukemia cells. Hypoxanthine at 5 mM also

reduced the zero-*trans* entry of 800 μM [^3H]adenine in L241 cells about 50%, but 5 mM thymidine had little effect (data not shown). These results are consistent with the view that adenine and hypoxanthine are transported by the same system, as suggested by several investigators (2, 7, 17). However, they cannot prove this point, and some of our results are not consistent with this conclusion, but suggest an alternate explanation of the data. For example, we have been unable in two independent experiments to detect any countertransport of adenine in ATP-depleted Novikoff cells either by adenine, hypoxanthine, or nucleosides (data not shown). Similar negative results were obtained in three independent experiments with untreated CHO DAP12 cells (data not shown). Furthermore, hypoxanthine and nucleosides have little, if any, effect on the zero-*trans* entry of adenine in Novikoff and

FIG. 5. Comparisons of initial time courses of zero-trans entry and exit and inward and outward equilibrium exchange of 5 mM adenine in L241 cells at 25°C. Changes in intracellular concentration of [3 H]adenine ($S_{2,t}$ or $N_{2,t}$) were measured in triplicate in four experimental protocols in the same population by using the rapid kinetic technique described in the text. The concentration of radioactivity was 208 cpm/ μ l. Equations 1, 3, and 4 (see text) were fitted to the data for zero-trans entry (Δ), and inward ($\blacktriangle$) and outward ($\blacktriangledown$) equilibrium exchange, respectively, with K ($=K_{ee}$) fixed at 3,000 μ M. The listed initial zero-trans and equilibrium exchange velocities (v_{12}^{zt} and v^{ee} in picomoles per microliter of cell water per s) were calculated for $S_1 = S = 5$ mM by substituting the estimated values for V and V^{ee} into: $v_{12}^{zt} = S_1 V / (K + S_1)$ and $v^{ee} = S V^{ee} / (K_{ee} + S)$, respectively. The curve for zero-trans exit of adenine was drawn by hand.

CHO cells, whereas hypoxanthine transport is strongly inhibited by nucleosides, but not by adenine (6). Only when CHO DAP12 cells were preincubated with KCN and iodoacetate was a slight countertransport of adenine by hypoxanthine apparent, but thymidine was as effective as hypoxanthine in stimulating the transient accumulation of radiolabeled adenine, whereas preloading the cells with unlabeled adenine had no significant effect (Fig. 7B).

FIG. 6. Zero-trans uptake of 80 to 5,120 μ M adenine by L241 cells at 24°C. The uptake of 80, 160, 320, 640, 1,280, 2,560, and 5,120 μ M [3 H]adenine (393 cpm/ μ l, irrespective of concentration) was determined by the rapid kinetic technique described in the text. Radioactivity per cell pellet was corrected for substrate trapped in extracellular space (2.3 μ l per pellet) and converted to picomoles per microliter of cell water on the basis of an intracellular water space of 10 μ l per pellet. Equation 1 was fitted to the pooled data, and the best-fitting parameters for K and V are listed in Table 2. Representative uptake time courses are illustrated in frames A, B, and C.

We also determined the effect of preloading cells with purines and nucleosides on the uptake of radiolabeled hypoxanthine. Compared with unlabeled hypoxanthine, adenine effected only a slight countertransport of radiolabeled hypoxanthine in either ATP-depleted L241 or hypoxanthine phosphoribosyltransferase-deficient Novikoff cells (Fig. 7C and D). Uracil preloading slightly stimulated hypoxanthine uptake in both types of cells. Thymidine had only a slight effect in L241 cells, like adenine and uracil (Fig. 7C), whereas in Novikoff cells it was as effective as hypoxanthine (Fig. 7D), an observation which agrees with the strong inhibition of hypoxanthine zero-trans entry by nucleosides in these cells mentioned already.

TABLE 2. Kinetic parameters of adenine transport by mouse L cells^a

Cell line	K (μ M)	V (pmol/ μ l of cell water per s)	Correlation coefficient ($r_{y,s}$)	k (V/K) (s^{-1})
L241	3,049 $\pm$ 231	91 $\pm$ 3	0.985	0.029
L929-2	3,932 $\pm$ 312	104 $\pm$ 4	0.988	0.026
Ltk ⁻	2,338 $\pm$ 337	150 $\pm$ 11	0.961	0.067
Ltk ⁻	3,445 $\pm$ 685	146 $\pm$ 17	0.954	0.042

^a The details of the experiment with L241 are given in the legend to Fig. 6. The experiments with the other cells were conducted in an identical manner.

FIG. 7. Countertransport of $10 \mu\text{M}$ $[^3\text{H}]$ adenine (A and B) and $10 \mu\text{M}$ $[^3\text{H}]$ hypoxanthine (C and D) in untreated L241 (A), ATP-depleted CHO-DAP12 (B), ATP-depleted L241 (C), and Novikoff cells deficient in hypoxanthine phosphoribosyltransferase (D). The cells were preloaded where indicated with 5 mM hypoxanthine (Hyp), thymidine (dThd), adenine (Ade), or uracil (Ura) at 37°C for about 10 min, and then their uptake of radiolabeled substrate was measured at 25°C as described in the text.

Overall, the results from countertransport and influx inhibition experiments cannot be explained satisfactorily on a mechanistic basis. In the case of nucleosides and hypoxanthine it seems most likely that different carriers transport these substrates in all cells, and that the substrates inhibit the transport of each other without being transported themselves by the system they inhibit. This view is supported by the finding that nucleoside and hypoxanthine transport in CHO cells clearly differ in their sensitivity to inhibition by nitrobenzylthionucleosides (20) and that a nucleoside transport-defective mutant of S49 mouse leukemia cells transports hypoxanthine and adenine normally (1). A similar mechanism could also account for the reciprocal inhibition of adenine and hypoxanthine transport by each other. Several investigators have found that adenine uptake by cells in which it is phosphoribosylated was inhibited by relatively low concentrations of hypoxanthine and concluded from this finding that both are transported by the same system (2, 7, 17). Such inhibition, however, may be due to competition for phosphoribosylpyrophosphate in phosphoribosylation rather than for a transporter. The question of the number of carriers involved in the transport of nucleobases and their substrate specificity can only be resolved unequivocally by the isolation and characterization of appropriate transport mutants or by the isolation

of the transporters and the assessment of their substrate specificities in reconstituted systems or by both.

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