Biochimica et Biophysica Acta 897 (1987) 431–444
Elsevier

BBA 73390

Nucleoside and nucleobase transport and metabolism in wild type and nucleoside transport-deficient *Aedes albopictus* cells

Tanveer F. Abidi [a], Peter G.W. Plagemann [b], Clive Woffendin [b] and Victor Stollar [a]

[a] *Department of Molecular Genetics and Microbiology, Robert Wood Johnson Medical School University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854 (U.S.A.) and* [b] *Department of Microbiology, Medical School, University of Minnesota, Minneapolis, MN 55455 (U.S.A.)*

(Received 27 October 1986)

Key words: Nucleoside transport; Adenine transport; Nucleoside non-mediated permeation; (Mosquito)

Nucleoside and nucleobase transport and metabolism were measured in ATP-depleted and normal *Aedes albopictus* mosquito cells (line C-7-10) by rapid kinetic techniques. The cells possess a facilitated diffusion system for nucleosides, which in its broad substrate specificity and kinetic properties resembles that present in many types of mammalian cells. The Michaelis-Menten constant for uridine transport at 28°C is about 180 μM. However, the nucleoside transporter of the mosquito cells is resistant to inhibition by nmolar concentrations of nitrobenzylthioinosine and the cells lack high affinity nitrobenzylthioinosine binding sites. The cells also possess an adenine transporter, which is distinct from the nucleoside transporter. They lack, however, a hypoxanthine transport system and are deficient in hypoxanthine phosphoribosyltransferase activity, which explains their failure to efficiently salvage hypoxanthine from the medium. The cells possess uridine and thymidine phosphorylase activities and, in contrast to cultured mammalian cells, efficiently convert uracil to nucleotides. An adenosine-resistant variant (CAE-3-6) of the C-7-10 cell line is devoid of significant nucleoside transport activity but transports adenine normally. Residual entry of various nucleosides into these cells and of hypoxanthine and cytosine into wild type and mutant cells is strictly non-mediated. The rate of permeation of various nucleosides and of hypoxanthine into the CAE-3-6 cells is related to their hydrophobicity. Uridine permeation into CAE-3-6 cells exhibits an activation energy of about 20 kcal/mol. At high uridine concentrations permeation is sufficiently rapid to partly overcome the limitation in nucleoside salvage imposed by the nucleoside transport defect in these cells.

Introduction

Various types of mammalian cells express only a single non-concentrative nucleoside transporter [1–3], which, however, exists in two forms [4–7]. Both forms exhibit similar kinetic properties and broad substrate specificity, but are distinguished on the basis of their sensitivity to inhibition by nitrobenzylthioinosine (NBTI); one system is inhibited by nmolar concentrations of NBTI and inhibition correlates with binding of NBTI to high-affinity binding sites on the transporter ($K_d \approx 0.5$ nM; designated NBTI-sensitive transport), whereas the other system lacks high affinity NBTI binding sites and is inhibited only by μmolar concentrations of NBTI (designated NBTI-resistant transport). In addition, Na^+-dependent, concentrative nucleoside transport has been de-

Correspondence: P.G.W. Plagemann, Department of Microbiology, Mayo Memorial Building, Box 196, 420 Delaware Street S.E., Minneapolis, MN 55455, U.S.A.

0005-2736/87/$03.50 © 1987 Elsevier Science Publishers B.V. (Biomedical Division)

tected in brush-border membranes of the kidney and intestine [8,9]. In mammalian cell lines, adenine and uracil each are transported by non-concentrative systems distinct from those transporting nucleosides or hypoxanthine [10,11]. In some cell lines, hypoxanthine is also transported by a system that is clearly different from that transporting nucleosides, but in other cell lines, nucleoside and hypoxanthine transport exhibit overlapping properties [7,12,13].

Little is known about the nucleoside and nucleobase transport systems of other vertebrates and of invertebrates. Sherwood and Stollar [14] have isolated an adenosine-resistant variant (CAE-3-6) from a line of *Aedes albopictus* mosquito cells (C-7-10), which exhibits an enhanced resistance to growth inhibition by a number of different nucleoside analogs and is defective in the uptake of 20 μM adenosine and thymidine. These results suggested that the mosquito cells possess a nucleoside transport system with a broad substrate specificity similar to that of the transporter of mammalian cells. In the present study, we have characterized the nucleoside and nucleobase transport systems of these cells and assessed the effect of a nucleoside transport deficiency on the ability of the cells to salvage nucleosides and nucleobases.

Experimental procedures

Cell culture. The clonally derived C-7 line of *Aedes albopictus* and its adenosine-resistant variant (CAE-3) have been described previously [14]. The C-7-10 and CAE-3-6 cells used in the present study are subclones of the C-7 and CAE-3 lines, respectively. All cells were propagated at 30°C in suspension culture in Eagle's minimal essential medium, which was supplemented with 10% (v/v) fetal bovine serum, non-essential amino acids, Pluronic F68 and additional 10 mM D-glucose as described for mammalian cell lines [15]. For experiments, cells were harvested from mid to late exponential phase cultures and suspended to (1–5) $\cdot 10^7$ cells/ml of serum free basal medium 42B (BM42B; Ref. 16). Cells were enumerated in a Coulter counter. Cultures of these cells were examined for mycoplasma contamination by culture methods, DNA staining and mycoplasma-mediated cytotoxicity of 6-methylpurine deoxyriboside [17]. No contamination was detected.

Transport and uptake of nucleosides and purines. 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. Nucleoside transport in ATP-depleted wild-type C-7-10 cells was measured in cell suspensions at 28°C as described previously [1,6,18,19]. Time-courses of transmembrane equilibration of substrate were determined under zero-*trans* and equilibrium exchange conditions [1] by rapid kinetic techniques (15 time points per time-course). Zero-*trans* 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 [1,20]. In experiments to determine Michaelis-Menten parameters, six substrate concentrations were used (40–1280 μM), and the parameters were extracted by least-squares regression. Initial velocities of equilibrium exchange were estimated by fitting the integrated rate equation for equilibrium exchange to the substrate equilibration curves [1,20]. Cells were depleted of ATP prior to transport measurements by incubation in glucose-free BM42B containing 5 mM KCN and 5 mM iodoacetate at 28°C for 20 min as described previously for mammalian cells [18,19].

Uptake of uridine, adenine and hypoxanthine by untreated wild type C-7-10 cells was measured in the same way and initial uptake velocities were estimated graphically from the initial linear portions of the uptake curves. For measuring effects of NBTI, dipyridamole and dilazep on uridine uptake, these were added to cell suspensions (at 28°C) at least 2 min prior to the uptake assay.

Longer time-courses of uptake of nucleosides and nucleobases were determined by supplementing samples of cell suspension with radiolabeled substrates and unlabeled nucleosides or inhibitors as indicated in appropriate experiments and sampling the suspensions manually during incubation at 28°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 [19].

In each experiment, values for radioactivity in cell pellets were corrected for substrate trapped in the extracellular space of cell pellets, which was estimated by the use of [^{14}C]inulin [19]. Concentrations of intracellular substrate equivalents were expressed on the basis of intracellular water space determined by the use of ^3H_2O [19]. The mean ($\pm$S.E.) values for the intracellular water space (in μl/10^7 cells) were 10.9 ± 0.8 ($n = 12$) for C-7-10 and 11.0 ± 0.7 ($n = 16$) for CAE-3-6 cells.

For fractionating the acid-soluble pool of labeled cells, samples of cells were centrifuged through an oil layer directly into 100 μl of a solution composed of 0.5 M trichloroacetic acid and 10% (w/v) sucrose, which rapidly quenches all metabolism [15,19]. To quantitate the various phosphorylated derivatives of uridine, the acid layer was further processed and analyzed by ascending paper chromatography with a solvent composed of 30 ml 1 M ammonium acetate (pH 5) and 70 ml 95% ethanol (solvent 28) as described previously [15,21]. For measuring uridine phosphorolysis by whole cells, the cells were separated from the medium by centrifugation through oil and the supernatants were chromatographed with a solvent composed of 86 parts of *n*-butanol and 14 parts H_2O (solvent 30) for the separation of uridine and uracil [21]. For measuring thymidine phosphorylysis, thymidine and thymine in cell supernatants were separated and quantitated by HPLC using reverse phase chromatography. Filtered samples were passed isocratically with 10 mM $(NH_4)_2HPO_4$ containing 10% (v/v) methanol through a Whatman Partisil ODS-3 column (4.5 $\times$ 250 mm) at 1.5 ml/min at 37°C. The effluent was monitored at 260 nm and fractions corresponding to thymidine and thymine were collected and analyzed for radioactivity.

Other methods. The octanol/water partition coefficients of nucleosides and purines were estimated as described previously [22] and hypoxanthine phosphoribosyltransferase activity in cell free extracts was measured as described by Fenwick and Casky [23].

Materials. [8-^{14}C]Adenine, [8-^{14}C]hypoxanthine, [5-^{3}H]uracil, [5-^{3}H]cytosine, [2,8-^{3}H]adenosine, [5-^{3}H]uridine, [2,8-^{3}H]deoxyadenosine, [*methyl*-^{3}H]thymidine and [5-^{3}H]deoxycytidine were purchased from Moravek Biochemicals (Brea, CA). Unlabeled nucleosides and nucleobases were obtained from Sigma Chemical Co. (St. Louis, MO) and unlabeled NBTI from Calbiochem (San Diego, CA). Dipyridamole, dilazep and deoxycoformycin were gifts from Geigy Pharmaceutical's (Yonkers, NY), Asta Werke (Frankfurt, F.R.G.), and Warner/Lambert (Park Davis Co., Detroit, MI), respectively.

Results and Discussion

Uptake and transport of uridine by wild-type C-7-10 cells

Fig. 1A shows time-courses of uptake of various concentrations of [^{3}H]uridine by C-7-10 cells measured by rapid kinetic techniques (the concentration of [^{3}H]uridine/ml of suspension was kept constant, while its specific radioactivity was decreased by addition of unlabeled uridine). At low concentrations of [^{3}H]uridine (10 and 30 μM), the intracellular radioactivity accumulated to concentrations exceeding those in the extracellular fluid by 40 s of incubation, whereas at 1 000 μM [^{3}H]uridine only equilibration with the intracellu-

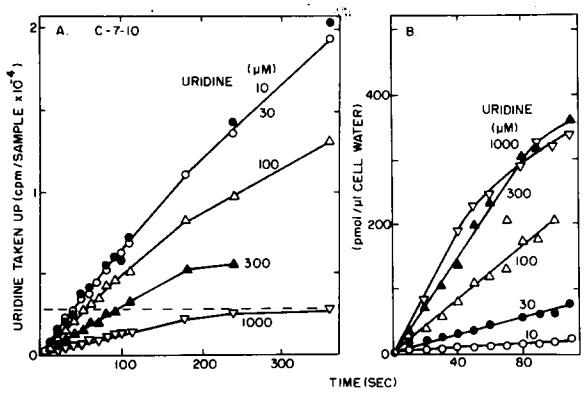

Fig. 1. Time-courses of uptake of various concentrations of uridine by C-7-10 cells. (A) The uptake of the indicated concentrations of [^{3}H]uridine (200 cpm/μl, irrespective of concentration) was measured in a suspension of $1.7 \cdot 10^7$ cells/ml by rapid kinetic techniques at 28°C as described in Experimental Procedures. Cell pellets were analyzed for radioactivity and the values corrected for substrate trapped in extracellular space. The broken line indicates the intracellular concentration of radioactivity equivalent to that in the medium at zero time. (B) The radioactivity values were converted to pmol/μl cell water on the basis of an experimentally determined cell water space.

lar space was observed. The saturation of uridine uptake above equilibrium concentrations clearly reflected saturation of uridine kinase. For example, after 360 s of incubation with 100 μM [^{3}H]uridine, 60% of the intracellular radioactivity was associated with UTP, 10% with UMP and 35% with unmodified uridine (see Fig. 2B), whereas after incubation with 1000 μM [^{3}H]uridine only about 10% of the intracellular radioactivity was associated with uracil nucleotides (data not shown).

Initial uptake was linear for at least 40 s at all uridine concentrations. The initial uptake velocities estimated from the linear portions of the uptake curves (Fig. 1B) probably represent fair estimates of the entry velocities, since they are measured at intracellular concentrations well below the equilibrium level [24]. A fit of the Michaelis Menten equation to these values yielded K_m and V_{max} values of 146 μM and 5.3 pmol/μl cell water per s, respectively (Table I).

In order to obtain uridine transport data that were not subject to uncertainties because of intracellular phosphorylation and thus trapping of the substrate, we resorted to ATP-depleted cells. Fig. 2B shows that preincubation of the C-7-10 cells in glucose-free medium containing 5 mM KCN and 5 mM iodoacetate at 28°C for 15 min almost completely prevented the phosphorylation of uridine entering the cells. The cells could be incubated in this medium for at least 1 h at 28°C without effect on the viability of the cells (measured by trypan blue exclusion), their intracellular water volume or their ability to transport uridine.

TABLE I

MICHAELIS-MENTEN PARAMETERS FOR THE TRANSPORT OF URIDINE IN C-7-10 CELLS

The details of the experiments are described in the legend to Fig. 1 and the text. The values in the first line are estimated from the data in Fig. 1 and those in lines 2 and 3 are from duplicate experiments. K, Michaelis-Menten constant; V, maximum velocity [1]. Values are the best fitting parameters ± S.E. of the estimate

Cells	K (μM)	V (pmol/μl cell H_2O per s)
Untreated	146 ± 43	5.3 ± 0.5
ATP-depleted	177 ± 18	15.9 ± 0.6
ATP-depleted	207 ± 12	10.2 ± 0.2

The initial rate of uptake of 100 μM uridine was about the same in ATP-depleted and untreated cells during the first 10 s of uptake (Fig. 2A). Thereafter, the uptake curves diverged reflecting the accumulation of uracil nucleotides in untreated cells and the equilibration across the membrane of unmodified uridine in ATP-depleted cells.

We have measured the zero-*trans* entry of uridine in ATP-depleted C-7-10 cells as a function of the uridine concentration and applied integrated rate analysis to the data for estimating the Michaelis-Menten parameters for zero-*trans* influx (Table I). The values obtained in two independent experiments were somewhat higher than those estimated from the initial rates of uptake of uridine in untreated cells.

The Michaelis-Menten parameters were computed on the basis of the simple carrier model assuming directional symmetry of the carrier and equal mobility of the empty and substrate-loaded carrier. These properties have been shown to pertain to the nucleoside carrier of mammalian cells

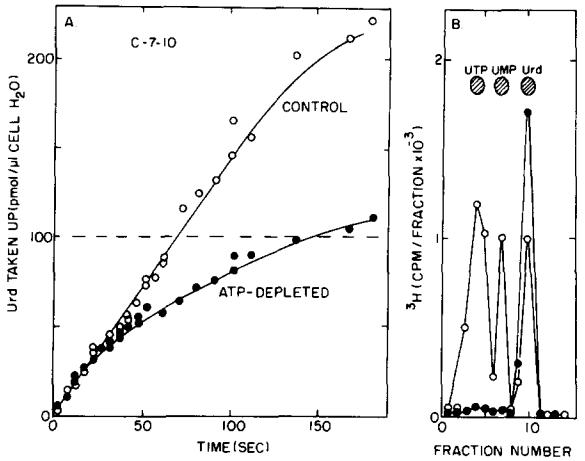

Fig. 2. Comparison of uptake of uridine by untreated and ATP-depleted C-7-10 cells. The cells were ATP-depleted and the uptake of 100 μM [^{3}H]uridine (5.9 cpm/pmol) was measured in untreated and ATP-depleted cells ($1 \cdot 10^7$ cells/ml) by rapid kinetic techniques at 28°C as described in Experimental Procedures. Radioactivity values were corrected for that trapped in extracellular spaces of cell pellets and converted to pmol/μl cell water on the basis of an experimentally determined cell water space. The broken line indicates the intracellular concentration of uridine equal to that in the medium. After 180 s of incubation, the acid-soluble pools were extracted from samples of cells and chromatographed with solvent 28 (B).

[1,20], but they also seem to apply to the carrier of the mosquito cells. This is indicated first by the excellent fit of the appropriate integrated rate equation to the time-courses of transmembrane equilibration of uridine at six concentrations (40–1 280 μM) under zero-*trans* conditions in ATP-depleted C-7-10 cells (see S.E. of estimate, Table I). Second, the initial velocities of zero-*trans* entry (v_{12}^{zt}) and inward equilibrium exchange (v^{ee}) at a uridine concentration (1 mM) well above the Michaelis-Menten constant were about equal (Fig. 3A). This coincidence is only observed when the mobility of empty and substrated-loaded carrier is equal [1]. The time-courses of zero-*trans* accumulation and inward equilibrium exchange of 1 mM uridine differed (Fig. 3A), which might imply differences in initial rates. But this difference is entirely predictable from the integrated rate equations for zero-*trans* influx and equilibrium exchange based on the simple carrier model [1]. In fact, the time-courses of [^{3}H]uridine accumulation in the zero-*trans* and equilibrium exchange modes correlated well with those simulated by computer for a simple carrier with directional symmetry and equal mobility when loaded or empty and with the kinetic parameters approximating those measured for the uridine transporter of these cells (Fig. 3B).

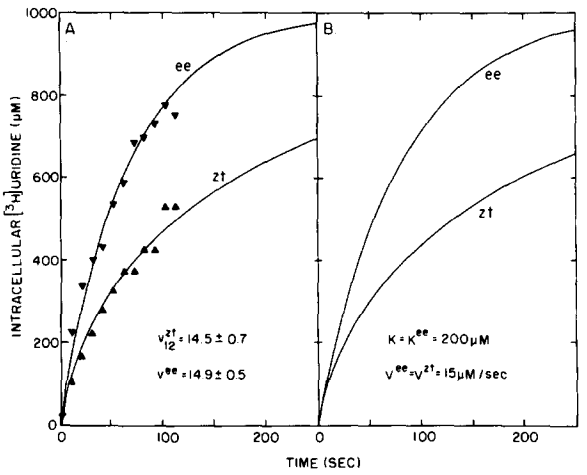

Fig. 3. Comparison of zero-*trans* influx and equilibrium exchange of uridine by ATP-depleted C-7-10 cells. (A) Time-courses of transmembrane equilibration of 500 μM [^{3}H]uridine (0.8 cpm/pmol) under zero-*trans* and equilibrium exchange conditions were measured in a suspension of $1 \cdot 10^7$ ATP-depleted C-7-10 cells per ml by rapid kinetic techniques at 28°C as described under Experimental Procedures. The initial velocities of zero-*trans* entry (v_{12}^{zt}) and equilibrium exchange (v^{ee}), which are expressed in pmol/μl cell water per s, were estimated by fitting the appropriate integrated rate equations [1] to the data with $K = K^{ee}$ fixed at 200 μM. (B) Simulated time-courses of zero-*trans* entry and inward equilibrium exchange for a simple transporter with directional symmetry and equal mobility when substrate-loaded or empty. The progress curves for intracellular substrate were generated for 500 μM extracellular substrate by numerical solutions of the integrated zero-*trans* entry and equilibrium exchange equations [1] with $K = K^{ee} = 200$ μM and $V = V^{ee} = 15$ μM/s.

Uridine uptake in C-7-10 cells was strongly inhibited by other nucleosides (Fig. 4A). The potency of inhibition by the various nucleosides at the same concentration (1 mM) was adenosine > thymidine > uridine > deoxycytidine. This effectiveness of inhibition resembles that observed for nucleoside transport in mammalian cells and reflects differences in affinity of the transporter for various nucleosides [1]. Hypoxanthine had no effect on uridine uptake (Fig. 3A). Uracil became inhibitory only after 30 s of incubation. This delayed effect is probably due to the conversion of uracil to uridine (see later) and the consequent lowering of the specific radioactivity of the substrate, rather than an effect on uridine transport, since the initial uptake rate was not affected.

Uridine transport in C-7-10 cells was also inhibited by 100 μM nifedipine, 50 μM dilazep and 50 μM dipyridamole, but not significantly by 1 μM NBTI and 50 μM lidoflazine (Fig. 4B). Of the substances investigated, dipyridamole was by far the most effective inhibitor of uridine transport. The concentration of dipyridamole causing a 50% inhibition of transport (IC_{50}) was estimated in another experiment to be about 4 μM (Fig. 4C).

Chromatographic analysis of the culture fluid of long term incubations (60–100 min) of C-7-10 cells with 100 μM [^{3}H]uridine (Fig. 5A) or 100 μM [^{3}H]thymidine (Fig. 5C) showed that a large proportion of the nucleosides in the medium became converted to uracil and thymine, respectively. In fact, the rate of accumulation of radioactivity by the cells decreased drastically between 10 and 20 min of incubation, while the appearance of the nucleobases in the medium continued unabated; thus after 100 min of incubation about 4 times more uridine had been converted to uracil and 20 times more thymidine had been converted to thymine than had become cell-associated.

Two points support the conclusion that the

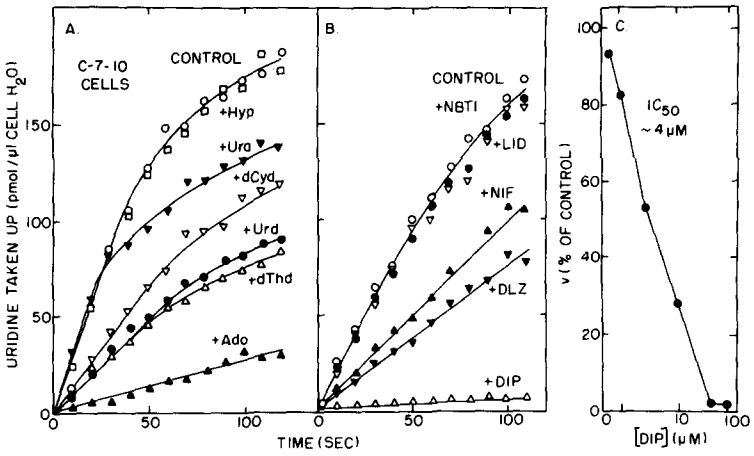

Fig. 4. Effect of other nucleosides and transport inhibitors on uridine uptake in C-7-10 cells. (A) The uptake of 200 μM [^{3}H]uridine (1.3 cpm/pmol) was measured in samples of a suspension of $1 \cdot 10^7$ cells/ml by rapid kinetic techniques as described in Experimental Procedures. Where indicated, unlabeled hypoxanthine (Hyp), uracil (Ura), deoxycytidine (dCyd), uridine (Urd), thymidine (dThd) or adenosine (Ado) were added simultaneously with radiolabeled uridine to final concentrations of 1 mM. (B) Samples of a suspension of $1.5 \cdot 10^7$ cells/ml were supplemented with 1 μM NBTI, 50 μM lidoflazine (LID), 100 μM nifedipine (NIF), 50 μM dilazep (DLZ) or 50 μM dipyridamole (DIP). After at least 2 min of incubation, the uptake of 100 μM [^{3}H]uridine (5.3 cpm/pmol) was measured as in (A). In (C) the uptake of 100 μM [^{3}H]uridine (5 cpm/pmol) was measured in samples of a suspension of $1.5 \cdot 10^7$ cells/ml that had been preincubated with the indicated concentrations of dipyridamole. Initial velocities of uptake were estimated from the initial linear portions of the uptake curves (see B) and are plotted as a function of the dipyridamole concentration.

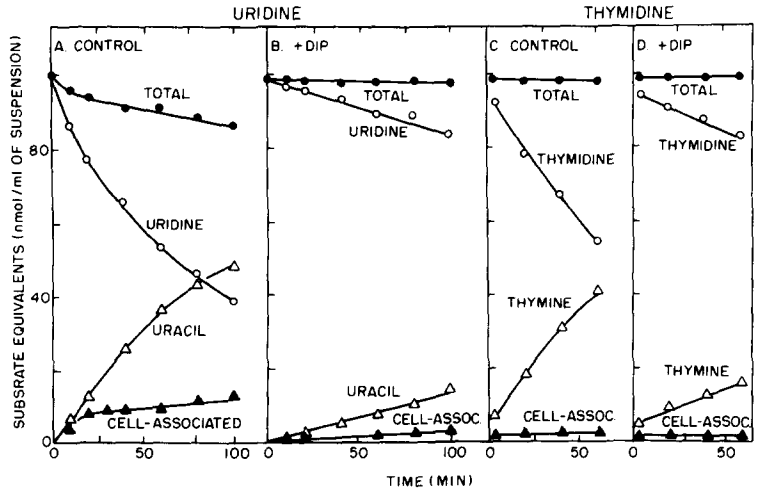

Fig. 5. Uridine and thymidine phosphorolysis in suspensions of C-7-10 cells. One sample (B and D) of suspensions of about $1 \cdot 10^7$ C-7-10 cells/ml was supplemented with 50 μM dipyridamole (DIP), whereas another sample remained untreated (A and C). DIP-treated and untreated suspensions were then supplemented as indicated with 100 μM [^{3}H]uridine (10 cpm/pmol) or 100 μM [^{3}H]thymidine (8 cpm/pmol). After various times of incubation at 28°C, the cells from samples of each suspension were collected by centrifugation through oil and analyzed for the amount of substrate taken up by the cells (cell-associated). The cell supernatant fluids remaining above the oil layer were analyzed for total radioactivity and chromatographed with solvent 30 to determine the proportion of radioactivity in uridine and uracil or by HPLC to determine the proportion of radioactivity in thymidine and thymine. The data are expressed as concentrations of nucleoside and nucleobase and the total of the two in the medium as well as the amount of cell-associated substrate equivalents per ml of cell suspension.

degradation of the nucleosides in suspensions of C-7-10 cells occurred intracellularly. First, inhibition of nucleoside uptake by dipyridamole also inhibited nucleoside degradation (Fig. 5B and D) and second, little, if any, uridine or thymidine became degraded during 100 min of incubation with the transport-deficient variant, CAE-3-6 (data not shown). The conversions of uridine and thymidine to their nucleobases were probably catalyzed by a single nucleoside phosphorylase. This conclusion is based on the observations first that the rates of phosphorolysis of uridine and thymidine by the C-7-10 cells were about the same and second that 1 mM unlabeled thymidine strongly inhibited the conversion of 100 μM [^{3}H]uridine to uracil by the cells (> 90%; data not shown). It should be emphasized that the amount of uridine that was converted to uracil during the first 1–2 min of incubation by C-7-10 cells was insignificant compared to the amounts accumulated by the cells; uridine phosphorolysis therefore was not an interfering factor in our uridine transport measurements with these cells.

Nucleoside uptake and metabolism in transport-deficient CAE-3-6 cells

The uptake of nucleosides by CAE-3-6 cells was very slow compared to nucleoside transport in wild type C-7-10 cells (Fig. 6A). For example, the rate of uptake of uridine at a concentration of 20 μM (about 0.01 pmol/μl cell water per s; Table II) was only about 1% of the rate of uridine transport in C-7-10 cells at this concentration (Fig. 1). However, the rate of uptake of the various nucleosides investigated varied greatly: from 0.167 to 0.0048 pmol/μl cell water per s for 20 μM adenosine and deoxycytidine, respectively (Table II). Except for adenosine, nucleoside uptake was approximately linear during the 80-min incubation period. The substrates accumulated intracellularly above the extracellular concentration and most of the intracellular radioactivity (ranging from about 50% for deoxycytidine to about 90% for adenosine) was associated with nucleotides. Only thymidine was less efficiently phosphorylated (data not shown).

The slow entry of the nucleosides into CAE-3-6-cells suggests that it was non-mediated. The following considerations support this conclusion but cannot prove it [1]. Lipid solubility is one of the primary factors determining the rate of non-mediated permeation of non-electrolytes through lipid bilayer membranes, but other factors such as the molecular size and shape of the permeant also play a role [25,26]. The relative rates of uptake of the nucleosides by CAE-3-6 cells were related, but not directly proportional, to their lipid solubility, which was assessed by their solubility in octanol as compared to that in water (Table II). However, the relationship between uptake rate and lipid

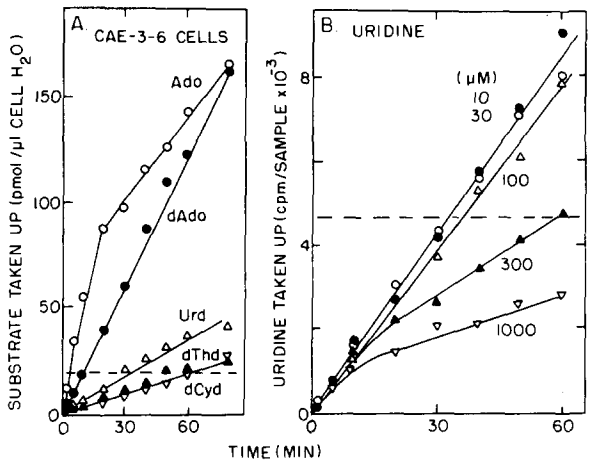

Fig. 6. Uptake of various nucleosides (A) and of various concentrations of uridine (B) by CAE-3-6 cells. (A) Samples of a suspension of $3.8 \cdot 10^7$ cells/ml were supplemented with 20 μM [^{3}H]adenosine (Ado; 160 cpm/pmol), [^{3}H]deoxyadenosine (dAdo; 500 cpm/pmol), [^{3}H]uridine (Urd; 630 cpm/pmol), [^{3}H]thymidine (dThd; 400 cpm/pmol) or [^{3}H]deoxycytidine (dCyd; 630 cpm/pmol). The suspensions receiving [^{3}H]adenosine or [^{3}H]deoxyadenosine were supplemented with 20 μM 2′-deoxycoformycin 5 min before addition of the radiolabeled substrate in order to prevent the deamination of the nucleosides. After various times of incubation at 28°C, the cells from 0.5-ml samples of suspension were collected by centrifugation through oil and analyzed for radioactivity. Radioactivity values were corrected for that trapped in extracellular space in cell pellets and converted to pmol/μl cell water on the basis of an experimentally determined water space. All values are averages of duplicate samples. The broken line indicates the intracellular concentration of substrate equal to that in the medium. (B) Samples of a suspension of $1.6 \cdot 10^7$ cells/ml were supplemented with the indicated concentrations of [^{3}H]uridine (630 cpm/μl, irrespective of concentration) and sampled as described in (A). The broken line indicates the intracellular concentration of radioactivity equal to that in the medium. After 60 min of incubation, the acid soluble pools were extracted from samples of cells and analyzed chromatographically (see Table III).

TABLE II

RELATIONSHIP BETWEEN RATES OF UPTAKE OF VARIOUS NUCLEOSIDES AND NUCLEOBASES BY CAE-3-6 CELLS AND THEIR OCTANOL/WATER PARTITION COEFFICIENTS

The initial velocities of uptake (v_o) were estimated from the initial linear portions of the uptake curves in Fig. 6A and from similar experiments with [^{3}H]hypoxanthine and [^{3}H]cytosine. The octanol/water partition coefficients were determined as described previously [22].

Substrate	v_o (at 20 μM) (pmol/μl cell H_2O per s)	K_{oct} (mean ± S.E.) ($n = 3$)	v_o/K_{oct}
Adenosine	0.167	0.161 ±0.004	1.0
2'-Deoxy-adenosine	0.0333	0.236 ±0.008	0.14
Uridine	0.0098	0.0149±0.0003	0.66
Thymidine	0.0052	0.753 ±0.002	0.073
2'-Deoxy-cytidine	0.0048	0.0272±0.002	0.18
Hypoxanthine	0.048	0.115 ±0.001	0.42
Cytosine	0.029	0.0352±0.00067	0.82

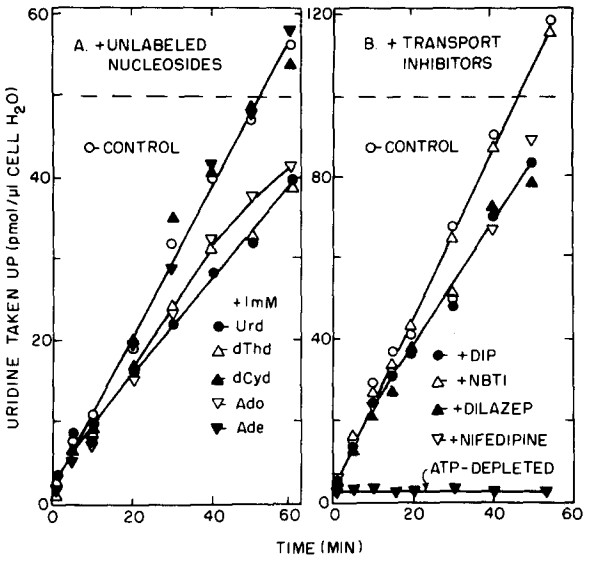

Fig. 7. Effects of other nucleosides (A) and transport inhibitors (B) on the uptake of uridine by CAE-3-6 cells. (A) Samples of a suspension of $1.9 \cdot 10^7$ cells/ml were supplemented with 50 μM [^{3}H]uridine (17 cpm/pmol) and simultaneously with 1 mM unlabeled adenosine (Ado), uridine (Urd), thymidine (dThd), deoxycytidine (dCyd) or adenine (Ade) and the uptake of [^{3}H]uridine was measured as described in the legend to Fig. 6. (B) Samples of a suspension of $2.4 \cdot 10^7$ cells/ml were supplemented with 40 μM dipyridamole (●), 100 μM dilazep (▲), 1 μM NBTI (△), or 100 μM nifedipine (▽) and then the uptake of 100 μM [^{3}H]uridine (9.4 cpm/pmol) was measured as in (A) in these and an untreated suspension (○). In addition, in (B) the cells of a sample of the suspension were depleted of ATP by incubation in glucose-free medium containing KCN and iodoacetate prior to measuring the uptake of 100 μM [^{3}H]uridine. The broken lines indicate the intracellular concentrations of substrate equal to those in the medium at zero time.

solubility for the various nucleosides (see v_o/K_{oct} values) was about the same as that observed for a nucleoside transport-deficient variant (AE_1) of the mouse lymphoma line S49 [27]. For both types of cells, the deoxynucleosides were less rapidly taken up in relation to their octanol partition coefficient than the ribonucleosides. What factor(s) accounts for this difference is unclear.

The conclusion that non-mediated permeation is the primary mode of entry of nucleosides into CAE-3-6 cells is further supported by the finding that, in contrast to nucleoside transport in C-7-10 cells, the entry of uridine into CAE-3-6 cells was non-saturable as judged by the initial uptake rates (Fig. 6B) and not significantly inhibited either by other nucleosides (Fig. 7A) or by transport inhibitors (Fig. 7B). The rate of uridine uptake by the cells decreased after 10–20 min of incubation when the uridine concentration was above 100 μM (Fig. 6B), but this decrease in uptake probably reflected effects on uridine phosphorylation and thus intracellular trapping, rather than saturation of uridine entry into the cells. At all uridine concentrations, most of the intracellular radioactivity, after 60 min of incubation, was associated with uracil nucleotides (Table III). The intracellular concentrations of unmodified uridine were much lower than those in the medium, which indicates that uridine entry into the cells was the rate determining step in its salvage by the cells. However, at higher concentrations, uridine probably entered the cells in sufficient amounts to support its phosphorylation at close to maximum rate resulting in nucleotide pool expansion and perhaps feedback inhibition of the kinase [24].

That the decrease in [^{3}H]uridine uptake with increase in absolute uridine concentration by CAE-3-6 cells (Fig. 6B) reflected limitations in phosphorylation rather than cell entry might have been assessed unequivocally by measuring uridine

TABLE III

INTRACELLULAR CONCENTRATIONS OF URACIL NUCLEOTIDES AND UNMODIFIED URIDINE OF CAE-3-6 CELLS AFTER 60 MIN OF INCUBATION WITH THE INDICATED CONCENTRATIONS OF [^{3}H]URIDINE AT 28°C

The details of the experiment are described in the legend to Fig. 6B. The intracellular concentrations of UTP, UMP and uridine (Urd) were calculated on the basis of the chromatographic analysis of the cell extracts and the total amounts of uridine (pmol/μl cell water) taken up by the cells. The uridine fraction contained any uracil (Ura) that may have been formed.

Extracellular uridine (μM)	Intracellular components (μM)		
	UTP	UMP	Urd (Ura)
10	7	5	0.6
30	28	30	3.3
100	55	67	7
300	168	137	7
1000	358	268	32

uptake in ATP-depleted cells. However, we could not use this approach because the depletion of CAE-3-6 cells of ATP by incubation in glucose-free medium containing KCN and iodoacetate completely inhibited uridine uptake by these cells (Fig. 7B). This finding has been confirmed in repeated experiments using [^{3}H]uridine concentrations ranging from 1 to 1000 μM. This effect clearly distinguishes uridine permeation in the nucleoside transport-deficient CAE-3-6 cells from nucleoside transport in wild type C-7-10 cells, which is not significantly affected by ATP depletion (Fig. 2). The mechanism of the inhibition of uridine permeation in CAE-3-6 cells by the KCN/iodoacetate treatment is not understood. It might be due to ATP depletion of the cells or a consequence of a direct effect of the chemical treatment on the structure of the plasma membrane.

Uridine uptake by the CAE-3-6 cells was highly temperature dependent (Fig. 8). The activation energy (20 kcal/mol) was similar to that for the non-mediated permeation of various substances (e.g., cytosine, azaguanine and L-glucose) into cultured mammalian cells [22]. However, such high activation energies do not distinguish between non-mediated permeation of substances through membranes and facilitated transport, since they are similarly high for both processes [1].

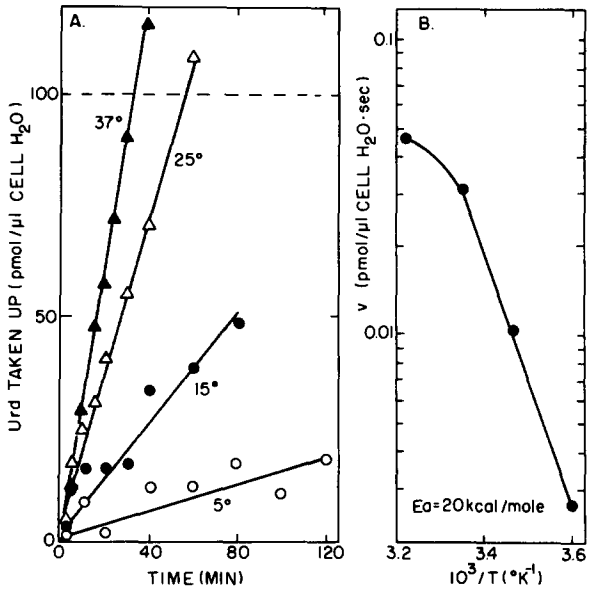

Fig. 8. Effect of temperature on the uptake of uridine by CAE-3-6 cells. (A) Samples of a suspension of $1 \cdot 10^7$ cells/ml were equilibrated at the indicated temperatures and then the uptake of 100 μM [^{3}H]uridine (10 cpm/pmol) was measured at these temperatures as described in the legend to Fig. 6. The broken line indicates the intracellular concentration of uridine equal to that in the medium. Velocities of uptake were estimated from the linear portions of the uptake curves and are plotted as a function of the absolute temperature in (B).

Lack of high affinity NBTI binding sites on C-7-10 and CAE-3-6 cells

When suspensions of about $2 \cdot 10^7$ C-7-10 or CAE-3-6- cells/ml were incubated with 1 nM [^{3}H]NBTI between 55% and 57% of the radioactivity became cell associated. This binding, however, was not affected by addition of unlabeled NBTI to a final concentration of 3 μM. This finding rules out the presence on these mosquito cells of high-affinity NBTI binding sites such as those observed in many types of mammalian cells [1–3,6,7].

Direct comparison of uridine uptake in C-7-10 and CAE-3-6 cells

When C-7-10 and CAE-3-6 cells were incubated with 5 μM [^{3}H]uridine, the former took up to 35-times more uridine and accumulated 39-times more [^{3}H]UTP than the latter in 60 min of incubation at 28°C (Fig. 9A; Table IV). The difference was much less at higher concentrations

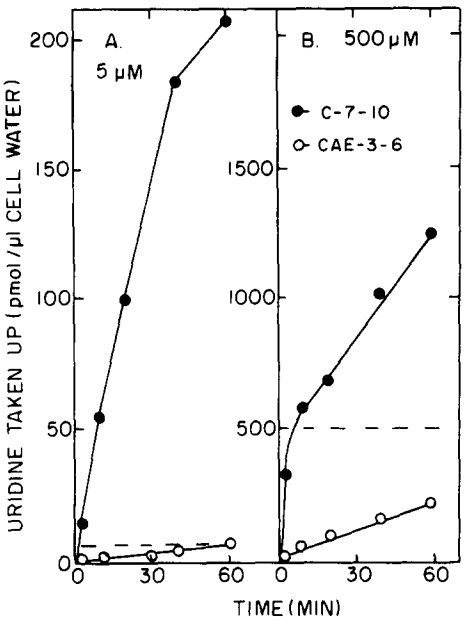

Fig. 9. Comparison of the uptake of 5 μM (A) and 500 μM (B) uridine by C-7-10 and CAE-3-6 cells. The uptake of [^{3}H]uridine (830 cpm/μl; irrespective of concentration) was measured in samples of suspensions of $2 \cdot 10^7$ cells/ml at 28°C as described in the legend to Fig. 6. All values are averages of duplicate samples and the broken lines indicate the intracellular concentrations of uridine equal to those in the medium at zero time. After 60 min of incubation, the acid soluble pools were extracted from samples of cells and analyzed chromatographically (see Table IV).

TABLE IV

COMPARISON OF INTRACELLULAR CONCENTRATIONS OF URACIL NUCLEOTIDES AND URIDINE IN C-7-10 AND CAE-3-6 CELLS AFTER 60 MIN OF INCUBATION WITH 5 OR 500 μM [^{3}H]URIDINE AT 28°C

The details of the experiment are described in the legend to Fig. 9. The intracellular concentrations of UTP, UMP and uridine (Urd) were calculated on the basis of the chromatographic analyses of the cell extracts and the total amounts of uridine taken up by the cells. The uridine fraction contained any uracil (Ura) that may have been formed.

Extracellular uridine (μM)	Intracellular components (μM)					
	C-7-10			CAE-3-6		
	UTP	UMP	Urd (Ura)	UTP	UMP	Urd (Ura)
5	147	56	6	3.8	1.8	0.4
500	710	210	280	170	77	<10

of uridine in the medium; at 500 μM [^{3}H]uridine, for example, the C-7-10 cells took up only 5-times more uridine and accumulated only 4-times more [^{3}H]UTP in 60 min of incubation than the CAE-3-6 cells (Fig. 9B; Table IV). This finding is explained as follows. At the low concentration in the medium, uridine is rapidly transported into C-7-10 cells and the entering uridine is efficiently phosphorylated, whereas in CAE-3-6 cells uridine phosphorylation is limited by the slow non-mediated permeation of uridine into the cells. At the higher uridine concentration, on the other hand, the capacity of the C-7-10 cells to transport uridine into the cells greatly exceeds their capacity to phosphorylate it (Fig. 9B), presumably due to a much higher K_m for uridine transport than for phosphorylation, just as is observed in mammalian cells [24]. In contrast, the transport deficiency in CAE-3-6 cells is partly overcome by increased non-mediated permeation when the uridine concentration in the medium is increased. Thus, in 60 min of incubation, C-7-10 cells formed only 5-times more UTP at 500 μM than at 5 μM extracellular [^{3}H]uridine, whereas the corresponding value for CAE-3-6 cells was 45 times (Table IV). A similar situation pertained for the uptake of 1 and 100 μM [^{3}H]thymidine by C-7-10 and CAE-3-6 cells (data not shown). This interaction in nucleoside salvage of transport and non-mediated permeation with intracellular phosphorylation needs to be taken into consideration when comparing nucleoside uptake by wild type and transport-deficient variants. This consideration is particularly important in the case of nucleosides, such as adenosine, which permeate membranes relatively rapidly due to their comparably high hydrophobicity (Table II).

Purine uptake by C-7-10 and CAE-3-6 cells

The uptake of adenine was very rapid and similar in the nucleoside transport-deficient and wild type cells (Fig. 10A and B). In fact, at a concentration of 500 μM adenine, the initial rate of adenine uptake was at least twice that of zero-*trans* entry of uridine in C-7-10 cells. Adenine uptake was not affected in either cell type by 2 mM uridine or 1 mM hypoxanthine, and only slightly reduced by 40 μM dipyridamole (Fig. 10C). In contrast to adenine uptake, the uptake of

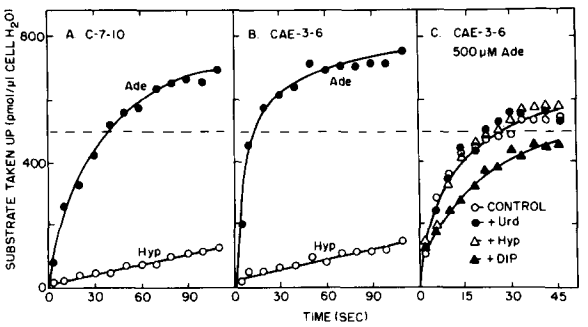

Fig. 10. Uptake of adenine and hypoxanthine by C-7-10 (A) and CAE-3-6 (B) cells and effect of uridine, hypoxanthine and dipyridamole on adenine uptake (C). (A and B) The uptake of 500 μM [^{14}C]adenine (0.23 cpm/pmol) and 500 μM [^{14}C]hypoxanthine (0.16 cpm/pmol) were measured in samples of suspensions of $2.0 \cdot 10^7$ C-7-10 and $2.4 \cdot 10^7$ CAE-3-6 cells/ml by rapid kinetic techniques as described in Experimental Procedures. (C) Uptake of 500 μM [^{14}C]adenine was measured in the same manner, except that, where indicated, 40 μM dipyridamole (DIP), 2 mM unlabeled uridine (Urd) or 1 mM unlabeled hypoxanthine (Hyp) were added simultaneously with the radiolabeled substrate. The broken lines indicate the intracellular concentrations of substrate equal to those in the medium.

hypoxanthine was very slow in both types of cells, only 1/250 of the initial rate of adenine uptake (Figs. 10A and B). In another experiment, hypoxanthine had not equilibrated across the plasma membrane in either cell type even after 80 min of incubation at 28°C, and the rate of uptake was directly proportional to the hypoxanthine concentration between 1 and 500 μM (data not shown). Also, hypoxanthine uptake was unaffected by 1.7 mM uridine (data not shown). The results indicate that these mosquito cells lack a hypoxanthine transport system and that hypoxanthine entry is solely non-mediated. The latter conclusion is also supported by the finding that the rate of entry of hypoxanthine into CAE-3-6 cells in relation to its octanol partition coefficient was of the same order of magnitude as observed for nucleoside permeation into these nucleoside transport-deficient cells (Table II).

The C-7-10 or CAE-3-6 cells also possessed little, if any, hypoxanthine phosphoribosyltransferase activity. First, only 10–50% of the intracellular radioactivity in both cell types after incubation with 4 μM [^{14}C]hypoxanthine for 60 min at 28°C was associated with nucleotides, whereas the proportion was > 80% after incubation with 4 μM [^{14}C]adenine. Second, the hypoxanthine phosphoribosyltransferase activity in cell-free extracts of C-7-10 cells was < 1% of that observed in comparable extracts of BHK_{21} cells used as controls.

A number of reports indicate that cultured cells of *Drosophila melanogaster* also fail to salvage hypoxanthine [28], but Becker [29] has reported that after propagation of *D. melanogaster* cells in a medium that is supplemented with glutamine, methotrexate, hypoxanthine or other purines and purine nucleosides, the cells do salvage hypoxanthine and hypoxanthine phosphorybosyltransferase activity becomes measurable in cell free extracts. These findings do not apply to the *A. albopictus* cells; the cells were routinely propagated in medium containing 2 mM glutamine and salvaged 5 μM [^{3}H]hypoxanthine only very slowly whether or not propagated for 1 day in medium containing 1 μM methotrexate or 200 μM hypoxanthine (data not shown).

Uracil uptake by C-7-10 and CAE-3-6 cells

When compared to uridine salvage, uracil salvage from the medium is very inefficient in mammalian cells whether (N1S1-67 cells) or not (P388 cells) they possess uridine phosphorylase (see Fig. 11C and D and Ref. 30); this is so even though the cells possess an uracil transport system [11]. This fact has been utilized for the detection of mycoplasma contamination in mammalian cell cultured [31]. Mycoplasma salvage uracil very efficiently because they possess a uracil phosphoribosyltransferase (see Ref. 32). Mammalian cells lack such an enzyme and uracil is a very poor substrate for orotate phosphoribosyltransferase at physiological pH (see Ref. 30). Conversion of uracil to UMP via the sequential action of uridine phosphorylase and uridine kinase is also very inefficient in those mammalian cells that possess uridine phosphorylase activity because of the limited availability of ribose 1-phosphate [30].

During routine checks for mycoplasma contamination using the uridine/uracil incorporation assay [31], we found that the mosquito cells took up uracil quite efficiently (Fig. 11A and B). In 30 min of incubation with 1 μM [^{3}H]uracil or 1 μM [^{3}H]uridine, the C-7-10 cells accumulated 20% as

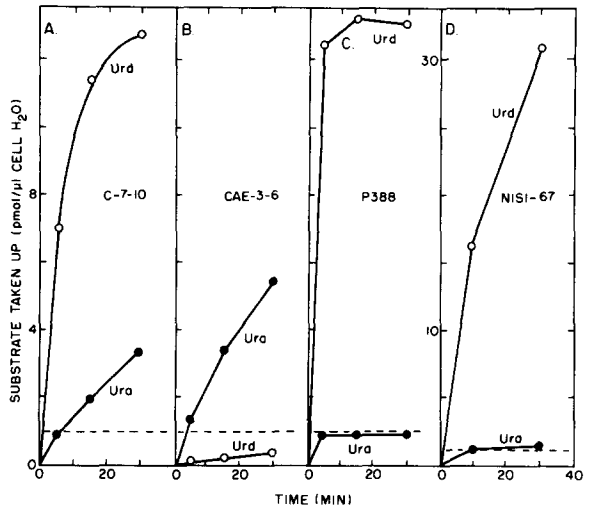

Fig. 11. Comparison of [^{3}H]uridine and [^{3}H]uracil uptake by C-7-10 and CAE-3-6 cells and mammalian cell lines that possess (Novikoff rat hepatoma cells, N1S1-67) or lack (P388) uridine phosphorylase activity [30]. Samples of suspensions of $4.5 \cdot 10^7$ C-7-10, $4.0 \cdot 10^7$ CAE-3-6, $3.6 \cdot 10^7$ P388 and $8 \cdot 10^6$ N1S1-67 cells/ml of BM42B were supplemented with 1 μM [^{3}H]uridine (1 000 cpm/pmol) or 1 μM [^{3}H]uracil/350 cpm/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 points represent averages of duplicate samples. The broken lines represent the intercellular substrate concentration equal to that in the medium at zero time. After 30 min of incubation, the acid soluble pools were extracted from samples of cells and chromatographed with solvent 28 for determining the amounts of radioactivity incorporated into uracil nucleotides (see Table V).

TABLE V

COMPARISON OF INTRACELLULAR CONCENTRATIONS OF URACIL NUCLEOTIDES BY MOSQUITO AND MAMMALIAN CELLS AFTER INCUBATION FOR 30 MIN WITH 1 μM [^{3}H]URIDINE OR 1 μM [^{3}H]URACIL AT 28°C

The details of the experiment are described in the legend to Fig. 11. The intracellular concentrations of UTP, UMP and uridine (Urd) or uracil (Ura) were calculated on the basis of the chromatographic analyses of the cell extracts and the total amounts of substrate taken up by the cells.

Cell line	Intracellular components (μM)					
	Uridine			Uracil		
	UTP	UMP	Urd	UTP	UMP	Ura
C-7-10	9.8	3.8	0.40	2.1	0.42	0.8
CAE-3-6	0.33	0.09	0.04	3.7	1.05	0.4
P388	11.3	1.5	0.24	<1	<1	0.9

much labeled UTP from uracil as from uridine (Table V). In contrast, the two mammalian cell lines tested accumulated uracil only to equilibrium levels (Fig. 11C and D) and intracellular conversion to uracil nucleotides was negligible (Table V and Ref. 30). The uracil salvage by the mosquito cells cannot be attributed to myoplasma contamination). Three independent assays for mycoplasma (see Experimental Procedures) were routinely negative. Absence of mycoplasma was also indicated by the slow uptake of uridine by the nucleoside transport mutant CAE-3-6 when compared to uracil uptake (Fig. 11B). Mycoplasma generally take up uridine about as rapidly as uracil because uridine becomes rapidly degraded to uracil by these organisms which is then phosphoribosylated [30]. It has not been ascertained yet whether the mosquito cells possess a uracil phosphoribosyltransferase or convert uracil to UMP via uridine. In a preliminary experiment, little, if any, [^{3}H]uracil became converted to [^{3}H]uridine when a suspension of $3 \cdot 10^7$ ATP-depleted C-7-10 cells/ml was incubated with 100 μM [^{3}H]uracil at 28°C for 60 min (data not shown).

Comparison of uracil and uridine uptake by the CAE-3-6 cells and of uracil uptake between the wild-type and nucleoside transport-deficient cells indicates, however, that the mosquito cells probably possess a uracil transport system and that uracil transport is distinct from nucleoside transport. In contrast, cytosine entered both the C-7-10 and CAE-3-6 cells only very slowly. Its rate of entry in relation to its octanol partition coefficient was of the same order of magnitude as observed for hypoxanthine permeation and for nucleoside permeation in CAE-3-6 cells (Table II). Cytosine permeation, therefore, was probably non-mediated, just as observed for mammalian cells [22].

Conclusion

Our results show that *A. albopictus* cells possess a nucleoside transporter, which in its kinetic properties and broad substrate specificity resembles that of mammalian cells [1]. The transporter is resistant to inhibition by nmolar concentrations of NBTI and thus is comparable to the NBTI-resistant transporter observed in many types of mammalian cells. However, the mosquito cells are

devoid of any NBTI-sensitive transporter, which commonly co-exists with NBTI-resistant transport in many mammalian cell types [5,6]. As is the case for certain mammalian cells [6,33,34], the NBTI-resistance of nucleoside transport in the mosquito cells correlates with the absence of high-affinity NBTI binding sites on the cells. Thus, the *A. albopictus* cells differ from a small group of cells that have been reported to express solely NBTI-resistant nucleoside transport, even though they possess high-affinity NBTI binding sites [35].

In addition, the *A. albopictus* cells, like mammalian cells, possess adenine and uracil transport systems, which are distinct from the nucleoside transporter. However, they lack a hypoxanthine transporter and are deficient in hypoxanthine phosphoryltransferase activity. This explains the earlier observation that these cells fail to efficiently salvage hypoxanthine from the medium [36]. We have observed that S49 mouse lymphoma cells also lack a hypoxanthine transport system [37] that is commonly found in mammalian cells [1,13]. In the S49 cells, however, hypoxanthine is transported, though very inefficiently, by the nucleoside transporter [37]. This is not the case in *A. albopictus* cells. That hypoxanthine permeation into the mosquito cells seems to be solely non-mediated is suggested by the observation that hypoxanthine uptake (1) is the same in wild-type cells and the nucleoside transport-deficient variant, (2) is non-saturable and (3) is not affected by the addition of nucleosides. This difference between *A. albopictus* and S49 cells does not seem to be related to the fact that nucleoside transport in the mosquito cells is NBTI-resistant, whereas that of S49 cells is mainly NBTI-sensitive [6,13], since the NBTI-resistant transport of nucleosides in Novikoff rat hepatoma cells is inhibited by hypoxanthine and, conversely, hypoxanthine transport is inhibited by uridine [6,13].

Also, as observed for mammalian cells, nucleosides enter the mosquito cells non-mediated presumably by diffusion through the lipid bilayer [25]. As expected on the basis of this assumption [25,26], the rate of diffusion of various nucleosides is partly related to their hydrophobicity. In fact, the relationship between the rates of diffusion of the various nucleosides into the transport-deficient CAE-6 cells was very similar to that observed in the nucleoside transport-deficient variant (AE_1) of the S49 cells [27]. With high concentrations of a nucleoside in the medium, non-mediated permeation of the nucleoside is sufficiently rapid in both types of cells to partly overcome limitations in its salvage resulting from a deficiency in or an inhibition of the nucleoside transport system.

Acknowledgements

We thank Laurie Erickson and John Erbe for excellent technical assistance, and Yvonne Guptill for valuable secretarial help. This work was supported by United States Public Health Service research grants GM24468, AM35211 and AI11290 and training grants CA09138 (C.W.) and CA09069 (T.A.).

References

1 Plagemann, P.G.W. and Wohlhueter, R.M. (1980) Curr. Top. Membranes Transp. 14, 225–330
2 Paterson, A.R.P., Kolassa, N. and Cass, C.E. (1981) Pharmacol. Ther. 12, 515–536
3 Young, J.D. and Jarvis, S.M. (1983) Biosci. Rep. 3, 309–332
4 Wohlhueter, R.M., Marz, R. and Plagemann, P.G.W. (1979) Biochim. Biophys. Acta 553, 262–283
5 Belt, J.A. (1983) Mol. Pharmacol. 24, 479–484
6 Plagemann, P.G.W. and Wohlhueter, R.M. (1984) Biochim. Biophys. Acta 773, 39–52
7 Plagemann, P.G.W. and Wohlhueter, R.M. (1985) Biochim. Biophys. Acta 816, 387–395
8 LeHir, M. and Dubach, V.C. (1984) Pflügers Arch. 401, 58–63
9 Schwenk, M., Hegazy, E. and Lopez del Pino, V. (1984) Biochim. Biophys. Acta 805, 370–374
10 Puziss, M., Wohlhueter, R.M. and Plagemann, P.G.W. (1983) Mol. Cell. Biol. 3, 82–90
11 Wohlhueter, R.M., McIvor, R.S. and Plagemann, P.G.W. (1980) J. Cell. Physiol. 104, 309–319
12 Plagemann, P.G.M. and Wohlhueter, R.M. (1982) Biochim. Biophys. Acta 688, 505–514
13 Plagemann, P.G.W. and Wohlhueter, R.M. (1984) J. Membrane Biol. 81, 255–262
14 Sherwood, A. and Stollar, V. (1982) Somatic Cell Genet. 8, 575–585
15 Plagemann, P.G.W. and Wohlhueter, R.M. (1983) J. Cell. Physiol. 116, 236–246
16 Plagemann, P.G.W. and Erbe, J. (1974) J. Cell. Physiol. 83, 321–336
17 McGarrity, G.J. (1982) Adv. Cell Culture 2, 99–131
18 Plagemann, P.G.W., Marz, R. and Erbe, J. (1976) J. Cell. Physiol. 89, 1–18
19 Wohlhueter, R.M., Marz, R., Graff, J. and Plagemann, P.G.W. (1978) Methods Cell Biol. 20, 211–236

20 Wohlhueter, R.M. and Plagemann, P.G.W. (1982) Biochim. Biophys. Acta 689, 249–260
21 Plagemann, P.G.W. (1971) J. Cell. Physiol. 77, 213–240
22 Graff, J.C., Wohlhueter, R.M. and Plagemann, P.G.W. (1977) J. Biol. Chem. 252, 4185–4190
23 Fenwick, R.G., Jr. and Caskey, C.T. (1975) Cell 5, 115–122
24 Wohlhueter, R.M. and Plagemann, P.G.W. (1980) Int. Rev. Cytol. 64, 171–240
25 Stein, E.D. (1986) Transport and Diffusion Across Cell Membranes, pp. 69–112, Academic Press, Inc., Orlando
26 Deuticke, B. (1977) Rev. Physiol. Biochem. Pharmacol. 78, 1–95
27 Plagemann, P.G.W. and Woffendin, C. (1987) Mol. Cell. Biol. 7, 160–166
28 Nash, D. and Henderson, J.F. (1982) Adv. Comp. Physiol. Biochem. 8, 1–51
29 Becker, J.L. (1978) Biochimie 60, 619–625
30 McIvor, R.S., Wohlhueter, R.M. and Plagemann, P.G.W. (1985) J. Cell. Physiol. 122, 397–404
31 Schneider, E.L., Stanbridge, E.J. and Epstein, C.J. (1974) Exp. Cell Res. 84, 311–318
32 McIvor, R.S., Wohlhueter, R.M. and Plagemann, P.G.W. (1983) J. Bacteriol. 156, 192–197
33 Cass, C.E., Kolassa, N., Uehara, Y., Dahlig-Harley, F., Harley, E.R. and Paterson, A.R.P. (1981) Biochim. Biophys. Acta 649, 769–777
34 Jarvis, S.M. and Young, J.D. (1982) J. Cell. Physiol. 324, 47–66
35 Paterson, A.R.P., Ng, C.Y.C., Adjei, A.A. and Jacobs, E.S. (1986) Pflügers Arch. 407 (Suppl. 1), S5
36 Malinoski, F. and Stollar, V. (1981) Antiviral Res. 1, 287–299
37 Plagemann, P.G.W. and Wohlhueter, R.M. (1985) Biochim Biophys. Acta 855, 25–32