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MEDIATED TRANSPORT OF NUCLEOSIDES BY HUMAN ERYTHROCYTES

SPECIFICITY TOWARD PURINE NUCLEOSIDES AS PERMEANTS

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SUMMARY

Transport of uridine and thymidine across the plasma membrane of human erythrocytes is mediated by a facilitated diffusion mechanism with broad specificity toward the base portion and narrow specificity toward the sugar portion of pyrimidine nucleosides. Specificity of this mechanism was further investigated by measuring efflux of radioactivity when erythrocytes containing radioactive uridine were incubated in medium containing purine nucleosides. Adenosine, guanosine, inosine, and arabinosyladenine accelerated uridine efflux and were therefore considered substrates for the transport mechanism. 6-Thioinosine, 6-thioguanosine, and several *S*-substituted 6-thiopurine ribonucleosides inhibited efflux of radioactive uridine. Adenine nucleosides with sugar moieties other than ribose or arabinose inhibited or had no effect on uridine efflux.

INTRODUCTION

Nucleosides are transported across the plasma membrane by facilitated diffusion in several mammalian cell types [1–5]. Passage of monosaccharides across the erythrocyte membrane is mediated by a similar non-concentrative mechanism[6]. In general, mechanisms of facilitated diffusion exhibit saturation kinetics, mediate exchange between internal and external permeant** molecules, and show dependence of unidirectional flux on the concentration of permeant at the opposite membrane face.

The chemical specificity of facilitated diffusion mechanisms can be examined using procedures based on accelerative exchange diffusion, a "*trans*" membrane

Abbreviations: arabinosyladenine, 6-amino-9-β-D-arabinofuranosylpurine; hydroxynitrobenzylthioguanosine, 2-amino-6-[(2-hydroxy-5-nitrobenzyl)thio]-9-β-D-ribofuranosylpurine; nitrobenzylthioinosine, 6-[(4-nitrobenzyl)thio]-9-β-D-ribofuranosylpurine; 6-thioinosine and 6-thioguanosine, the 6-thio and 2-amino-6-thio derivatives, respectively, of 9-β-D-ribofuranosylpurine.

* Throughout this paper, "permeant" refers to compounds that are transported across biological membranes by mediated processes.

** In this work, the half-saturation constant for accelerative exchange diffusion of unlike permeants is defined as that concentration of extracellular permeant at which efflux is one-half maximal[3].

effect[3,6]. In accelerative exchange diffusion, the rate of transport of permeant in one direction is increased by transport from the opposite membrane face of the same or of a related permeant; acceleration of transport of permeant from the *cis* membrane face by permeant at the *trans* membrane face indicates that the accelerating and accelerated permeants are transported by the same mechanism.

Previous studies have suggested that transport of pyrimidine nucleosides in human erythrocytes is mediated by a non-concentrative mechanism with broad permeant specificity[1,3]. Experiments with radioactive uridine and thymidine, which are not metabolized by human erythrocytes[1], indicated that ribo- and 2′-deoxyribonucleosides with variously substituted pyrimidine moieties were readily accepted as permeants, whereas pyrimidine nucleosides with derivatized ribosyl or 2′-deoxyribosyl groups were poor substrates for the transport mechanism[3].

In the present study, accelerative exchange diffusion of uridine was used to examine the specificity of the nucleoside transport mechanism toward purine nucleosides. To determine whether a particular nucleoside was a substrate for the transport mechanism, efflux of uridine from uridine-loaded erythrocytes was measured in the presence of extracellular test nucleoside. Compounds that accelerated uridine efflux were thereby recognized as substrates for the transport mechanism, whereas compounds that reduced rates of outward transport of uridine were considered inhibitors of the transport mechanism. Adenosine, guanosine, and inosine accelerated uridine efflux, and of 12 adenine nucleosides with sugar moieties other than ribose, only arabinosyladenine accelerated efflux of radioactive uridine.

MATERIALS AND METHODS

The procedure for measuring efflux of radioactivity from human erythrocytes "loaded" with labeled uridine has been described previously[3]. Erythrocytes were obtained from whole blood (Red Cross Blood Transfusion Service, Edmonton, Alberta) after 21–28 days of storage at 4 °C in acid citrate–dextrose solution A (U.S.P.). Experiments using freshly collected blood showed that effects of storage for periods as long as 24 days on rates of uridine transport were not significant (C. E. Cass and A. R. P. Paterson, unpublished). Erythrocytes were washed 3 times in buffered saline (140 mM NaCl, 1.4 mM $MgSO_4$ and 18 mM *N*-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid at pH 7.4) with a final centrifugation at 1700 × *g* for 15 min; cell sediments prepared in this way had an extracellular space of 7–11% (ref. 1). To load cells with 6 mM [2-^{14}C]uridine or [5-^{3}H]uridine, measured volumes of erythrocyte sediments were incubated with equal volumes of buffered saline containing ~0.5 mM radioactive uridine for 40 min at 37 °C, conditions sufficient to obtain equal internal and external concentrations of uridine. Incubations were terminated by centrifugation (1700 × *g*, 15 min), and extracellular radioactivity was assumed to be a measure of the intracellular concentration of uridine.

Since the initial rate of outward flow of radioactive uridine at 25 °C was independent of extracellular volume when cell suspensions of 10% or less were used[3], efflux was assayed at 25 °C using 8–10% suspensions of erythrocytes and, unless otherwise noted, the following procedure was used. Outward movement of radioactivity was measured from cells loaded with 5.5–6.5 mM radioactive uridine;

the assay was initiated by rapidly adding to 0.25 ml portions of loaded cells 2.5 ml volumes of (1) buffered saline, (2) buffered saline containing the equilibrium concentration (5.5-6.5 mM) of non-radioactive uridine, or (3) buffered saline containing test compound Radioactive uridine originally present in the extracellular space of loaded cell sediments was diluted approx. 100-fold in this procedure. Uridine efflux was terminated after timed intervals by adding 2.5 ml buffered saline containing 50 μM hydroxynitrobenzylthioguanosine, a potent inhibitor of nucleoside transport[3]. Zero time points were obtained by adding hydroxynitrobenzylthioguanosine 10 s before addition of test medium. Separate reaction mixtures were prepared for each time point, and immediately after termination of efflux, portions of each mixture were centrifuged with di-1-butylphthalate (1700×*g*, 1.5 min) to obtain cell-free medium[1,7]. Radioactivity present in cell-free medium was assayed using Bray's solution[8] and liquid scintillation counting. The time course of appearance of radioactivity in the medium was determined at 5 or 10-s intervals, and straight lines were fitted to the data by the method of least squares. The cell concentrations of individual reaction mixtures were obtained from hematocrits determined by the capillary method.

To determine whether significant metabolism of adenosine occurred during transport experiments, cells loaded with 6 mM non-radioactive uridine were suspended in 10 volumes of buffered saline containing 6 mM [8-^{14}C]adenosine. Cell suspensions were incubated at 25 °C, and at timed intervals, 100-μl samples were added to tubes containing 5 μl of 42% $HClO_4$ at 4 °C. After 15 min, samples were neutralized at 4 °C by addition of an equivalent amount of KOH. The samples were then centrifuged and 10-μl portions of the supernatants were chromatographed with appropriate carrier bases, nucleosides, and nucleotides using the two-dimensional thin-layer system of Crabtree and Henderson[9]. Radioactivity was determined by direct counting of chromatogram sections in a liquid scintillation system. The counting solution consisted of 4 g 2,5-diphenyloxazole and 0.1 g 1,4-bis-2-(5-phenyloxazolyl)benzene per l of toluene.

6-Benzyl-2-amino-9-β-D-ribofuranosylpurine was a gift from Dr M. J. Robins, Dept of Chemistry, University of Alberta, Edmonton, Alberta. 6-Thioinosine, 6-thioguanosine, arabinosyladenine, 9-β-D-lyxofuranosyladenine, 2'-deoxy-2'-thiomethyladenosine, 3'-deoxy-3'-methyladenosine, 4-β-D-ribofuranosyl-5,7-dionethiazaolo-[5,4-d]pyrimidine, 8-methoxyadenosine, 9-β-D-6'-deoxyallofuranosyladenine, 3'-deoxy-3'-(2-hydroxyethyl)adenosine, 9-β-L-ribofuranosyladenine and 9-β-L-fucopyranosylhypoxanthine were generously provided by Drug Research and Development, National Cancer Institute, Bethesda, Maryland. Hydroxynitrobenzylthioguanosine and nitrobenzylthioinosine were prepared by Raylo Chemicals Ltd, Edmonton, Alberta. Other compounds were obtained from commercial sources.

RESULTS

When efflux was measured from cells loaded with different concentrations of uridine and incubated in medium without uridine (the "zero-*trans*" experiment of Karlish *et al.*[30]), the apparent half-saturation constant obtained from reciprocal plots of efflux *versus* intracellular uridine was 0.25 mM (C.E. Cass and A.R.P. Pater-

son, unpublished). The dependence of uridine efflux on the presence of nucleosides at the external membrane face was therefore studied by measuring outward fluxes from cells loaded with 6 mM uridine, a concentration sufficient to saturate the transport mechanism at the internal membrane face.

When measured from cells containing 6 mM uridine, efflux is half-maximal when the extracellular uridine concentration is 0.1 mM and is maximal at concentrations of 4 mM or greater[3]. In the experiments reported here, the mean rate (± standard deviation) for 33 separate determinations of uridine efflux from 8–10 % suspensions of cells loaded with 6 mM non-radioactive uridine and incubated in 6 mM non-radioactive uridine was 4.9 ± 0.6 μmoles/min per ml packed cells and in buffered saline was 1.1 ± 0.3 μmoles/min per ml packed cells. These determinations used blood obtained from 11 different individuals. Since radioactive uridine was present in the extracellular space of loaded packed cells. the initial concentration of extracellular uridine after addition of 2.5 ml buffered saline to 0.25 ml packed cells was about 0.06 mM.

The data of Fig. 1 demonstrate that extracellular adenosine accelerated efflux of intracellular radioactive uridine. Results are presented for 3 of 5 concentrations (0.1–6.0 mM) of adenosine that were examined; linear time courses were obtained at all concentrations. When efflux from cells loaded with 5.8 mM uridine was measured without adenosine in the medium (data not shown in Fig. 1), rates were 0.9, 1.1, and 1.1 μmoles/min per ml packed cells. Efflux was enhanced by addition of adenosine to the medium; when extracellular adenosine was 0.1–6.0 mM, rates were 1.4–1.9 μmoles/min per ml packed cells, or 34–48% of rates observed in the presence of 6 mM extracellular uridine. Stimulation of uridine efflux by adenosine appeared to be near maximal at 0.1 mM, suggesting that the apparent half-saturation constant for extracellular adenosine is somewhat lower than that previously observed (0.1 mM) for extracellular uridine[3].

In human erythrocytes, adenosine may be rapidly deaminated or phosphorylated[10,11]; conversion of adenosine to adenine by purine nucleoside phosphorylase occurs at a very low rate[12,13]. To determine whether metabolism of adenosine during measurements of uridine efflux was sufficient to have influenced results of the experiments of Fig. 1, erythrocytes loaded with 6 mM non-radioactive uridine were incubated with [8-^{14}C]adenosine under conditions used for assay of uridine efflux. Fig. 2 illustrates the time course of adenosine metabolism under conditions similar to those of the experiments of Fig. 1.

The major route of adenosine metabolism under the conditions of measurement of uridine efflux was deamination to inosine. At the end of the 2-h incubation in the experiment of Fig. 2, radioactivity from [8-^{14}C]adenosine had the following distribution: 64%, adenosine; 26%, inosine; 3%, hypoxanthine; 3%, guanine; 2%, adenine; 1%, guanosine; and 1%, nucleotides. In this experiment, the initial rates of adenosine disappearance and inosine formation were 0.14 and 0.12 μmole/min per ml packed cells, respectively; in 2 similar experiments, the initial rates of adenosine disappearance were 0.15 and 0.19 μmole/min per ml packed cells and of inosine formation were 0.10 and 0.15 μmole/min per ml packed cells. During the first 30 s of incubation with radioactive adenosine, the maximum possible intracellular concentration of inosine (0.06 mM) was small relative to initial concentrations of intracellular uridine (6 mM) and extracellular adenosine (6 mM). (The maximum internal concentration was calculated assuming that all newly

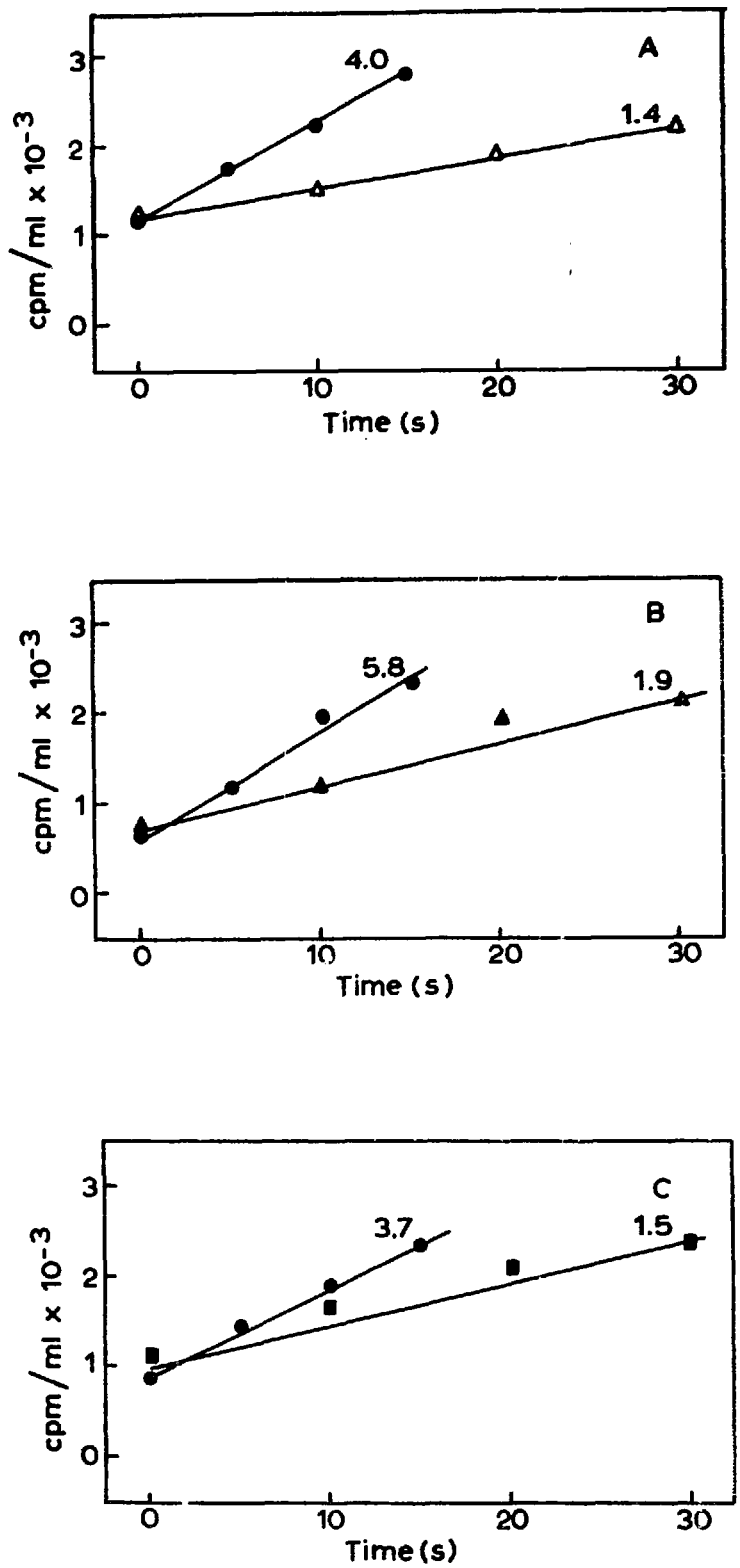

Fig. 1. Acceleration of uridine efflux by adenosine. Erythrocytes loaded with 5.8 mM [2-^{14}C]-uridine were incubated in buffered saline containing 5.8 mM non-radioactive uridine (●); 0.1 mM adenosine (△); 0.5 mM adenosine (▲); and 6.0 mM adenosine (■). Efflux of radioactivity was measured as described in Materials and Methods. Uridine efflux, expressed as μmoles/min per ml packed cells, is indicated for each time course of appearance of radioactivity in the medium; adenosine-accelerated efflux was 35%, 43% and 41% of uridine-accelerated efflux in Panels A, B, and C, respectively.

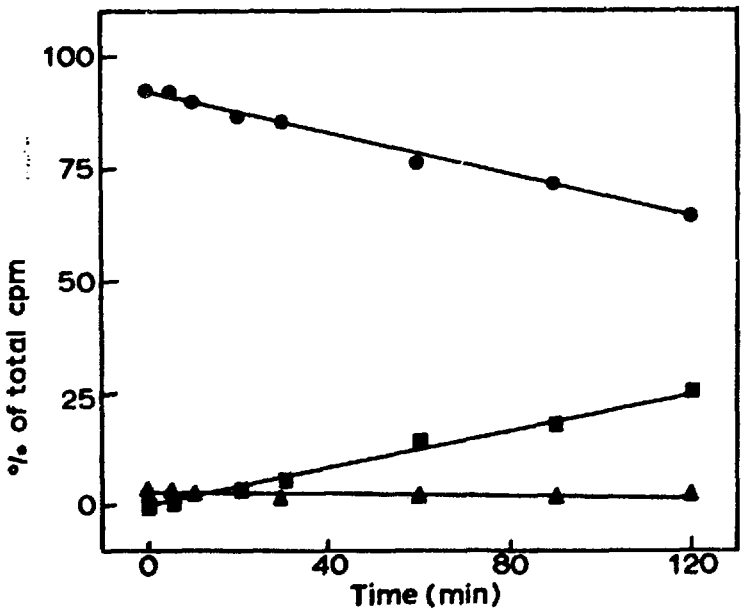

Fig. 2. Adenosine metabolism during adenosine-accelerated efflux of uridine. 50% suspensions of erythrocytes in medium containing 10.5 mM non-radioactive uridine were incubated for 40 min at 37 °C; cells had a final intracellular uridine concentration of 6.0 mM. 10% suspensions of the loaded cells were then incubated at 25 °C in buffered saline containing 6.5 mM [8-^{14}C]-adenosine ($4.5 \cdot 10^5$ cpm/μmole). At timed intervals, duplicate 100-μl samples were removed and prepared for chromatographic analyses (see Materials and Methods). Percentages of the total radioactivity found in the following compounds are plotted against time: ●, adenosine; ■ inosine; and ▲, hypoxanthine.

formed inosine was intracellular; intracellular water was approx. 75% of total cell volume[1].)

The acceleration of uridine efflux by several purine nucleosides is shown in Table I. After loading cells with 5.5–6.0 mM [2-^{14}C]uridine, efflux of radioactivity

TABLE I

ACCELERATION OF URIDINE EFFLUX FROM URIDINE-LOADED ERYTHROCYTES BY EXTRACELLULAR NUCLEOSIDES

Medium		*Uridine efflux*	
Added nucleoside	*mM*	*µmoles/min per ml packedcells*	*% of rate into uridine*
Uridine	5.5	4.9	100
Adenosine	7.1	2.4	49
Guanosine	4.4	3.0	61
None		1.0	20
Uridine	6.1	4.2	100
Inosine	5.5	2.7	64
None		1.6	38
Uridine	5.8	5.4	100
Arabinosyladenine	4.0	2.2	41
None		1.1	20
Uridine	6.0	5.3	100
4-β-D-Ribofuranosyl-5,7-dione-thiazaolo-[5,4-d]pyrimidine	6.0	3.8	72
None		1.0	19

was determined when cells were incubated in buffered saline containing (1) no additions, (2) 5.5–6.0 mM non-radioactive uridine, or (3) test nucleoside at the concentration indicated. When observed rates were expressed as percentages of uridine-accelerated efflux, values ranged from 40–71%; values obtained for efflux into buffered saline were approx. 20% of uridine-accelerated efflux.

The effect on uridine efflux of various concentrations of extracellular inosine was examined using a modification of the procedure described in Materials and Methods. After loading cells with 6 mM [5-^{3}H]uridine, the efflux assay was initiated by the rapid addition to 0.1 ml packed cells of 10 ml of (1) buffered saline, (2) buffered saline with 6 mM non-radioactive uridine, or (3) buffered saline with 0.01–6.0 mM inosine. Radioactive uridine present in the extracellular space of packed, loaded cells was thereby diluted to approx. 0.006 mM, a concentration below the half-saturation constant (0.1 mM) previously observed for extracellular uridine[3]. Cell concentrations of individual reaction mixtures were determined with a Model F Coulter Counter. Results from one such experiment are presented in Table II. Values for the concentration of extracellular inosine at which uridine efflux was one-half maximal were obtained from plots of reciprocals of uridine efflux and the extracellular concentrations of inosine; in 3 separate experiments, the apparent half-saturation constants for inosine were 0.004, 0.02, and 0.06 mM.

Six purine nucleosides had no effect on uridine efflux when tested as in Table I. Efflux of radioactive uridine into media containing test nucleoside was not significantly different from efflux into buffered saline for the following compounds, which were tested at the concentrations indicated: 2′-deoxyadenosine (5.3 mM), 3′-deoxyadenosine (0.48 mM), 8-azaadenosine (6.1 mM), 9-β-D-xylofuranosyladenine (1.5 mM), 9-β-D-lyxofuranosyladenine (1.4 mM) and 9-β-L-fucopyranosylhypoxanthine (6.1 mM).

15 purine nucleosides inhibited efflux of radioactive uridine. Inhibition was indicated by fluxes into medium containing test compound that were lower than

TABLE II

ACCELERATION OF URIDINE EFFLUX FROM URIDINE-LOADED ERYTHROCYTES BY EXTRACELLULAR INOSINE

Medium		*Uridine efflux* *	
Added nucleoside	*mM*	*μmoles/min per ml packed cells*	*% of rate into uridine*
Uridine	6.1	4.16	100
Inosine	0.043	1.82	44
	0.43	2.85	69
	5.51	2.66	64
None		1.62	39
Uridine	6.1	5.05	100
Inosine	0.079	2.35	47
	0.21	2.71	54
	1.71	2.48	49
None		1.75	35

* Data obtained on 2 consecutive days using blood from a single individual.

fluxes into buffered saline alone. Such results are illustrated in Fig. 3 for 6-thioguanosine and 3′-deoxy-3′-methyladenosine and are summarized in Tables III and IV; ribofuranosides with various bases and adenine nucleosides that differ in the sugar moiety are listed in Tables III and IV, respectively.

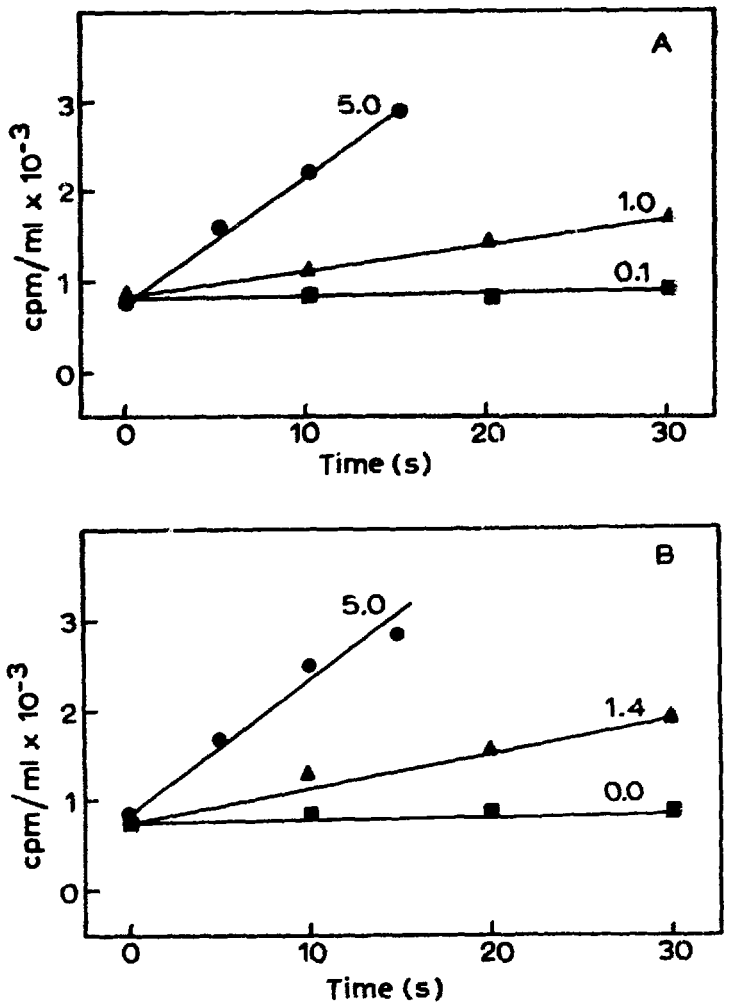

Fig. 3. Inhibition of uridine efflux by 6-thioguanosine and 3′-deoxy-3′-methyladenosine. Erythrocytes loaded with [2-^{14}C]uridine (5.6 mM in Panel A; 5.7 mM in Panel B) were incubated in buffered saline with or without added nucleoside and efflux of radioactivity was measured as described in Materials and Methods. Efflux (expressed as μmoles/min per ml packed cells) is indicated for each time course of outflow of radioactive uridine. Panel A: ●, 5.6 mM uridine; ▲, buffered saline; and ■, 1.4 mM 6-thioguanosine. Panel B; ●, 5.7 mM uridine; ▲, buffered saline; and ■, 6.4 mM 3′-deoxy-3′-methyladenosine.

12 inhibitory compounds were further tested by examining their abilities to inhibit the acceleration of uridine efflux by 6 mM extracellular uridine (Table V). The procedure is illustrated in Fig. 4 where 2′-deoxy-2′-thiomethyladenosine and 6-thioguanosine were assessed for inhibitory activity. Efflux from cells loaded with radioactive uridine was initiated by the addition of medium containing non-radioactive uridine. After 10 s, buffered saline containing (1) no additions, (2) test nucleoside, or (3) the transport inhibitor hydroxynitrobenzylthioguanosine was added to reaction mixtures. Hydroxynitrobenzylthioguanosine was added to stop [2-^{14}C]-uridine outflow, thereby providing a basal value for ^{14}C-content of the medium at

TABLE III

INHIBITION OF URIDINE EFFLUX FROM URIDINE-LOADED ERYTHROCYTES BY EXTRACELLULAR PURINE RIBONUCLEOSIDES

Medium		*Efflux (μmoles/min per ml packed cells) into:*		
Additive	*mM*	*Nucleoside*	*Uridine*	*Buffered saline*
6-Thioguanosine	1.4	0.1	5.0	1.0
6-Thioinosine	5.8	0.5	4.7	1.0
6-(Methylthio)-9-β-D-ribofuranosylpurine	6.0	0.4	5.3	0.9
Nitrobenzylthioinosine	0.001	0.2	5.1	
Hydroxynitrobenzylthioguanosine	0.006	0.1	5.5	
6-Benzyl-2-amino-9-β-D-ribofuranosylpurine	0.05	0.0	5.2	1.2
8-Methoxyadenosine	2.4	0.4	5.3	1.0

TABLE IV

INHIBITION OF URIDINE EFFLUX FROM URIDINE-LOADED ERYTHROCYTES BY EXTRACELLULAR ADENINE NUCLEOSIDES

Medium		*Efflux (μmoles/min per ml packed cells) into:*		
Nucleoside	*mM*	*Nucleoside*	*Uridine*	*Buffered saline*
9-β-D-6′-Deoxyallofuranosyladenine	5.2	0.0	4.8	0.9
3′-Deoxy-3′-(2-hydroxyethyl)adenosine	6.1	0.0	4.8	0.9
3′-Deoxy-3′-methyladenosine	6.4	0.0	5.0	1.4
2′-Deoxy-2′-thiomethyladenosine	6.6	0.0	5.0	1.4
2′, 3′-*O*-Isopropylideneadenosine	3.9	0.1	4.0	
9-β-D-Psicofuranosyladenine	6.3	0.3	4.8	1.2
9-β-L-Ribofuranosyladenine	2.1	0.0	4.8	1.1

TABLE V

INHIBITION OF URIDINE-ACCELERATED EFFLUX FROM URIDINE-LOADED ERYTHROCYTES BY EXTRACELLULAR PURINE DERIVATIVES

Efflux from cells loaded with 6 mM [2-^{14}C]uridine was determined as described in Fig. 4. In the following calculation, A, B and C represent reaction mixtures containing, respectively, no test compound, hydroxynitrobenzylthioguanosine (added at 10 s), or test compound at the concentration indicated beneath. % Inhibition =

$$100 - \left(\frac{\text{cpm in C at } T_{70}\text{ s} - \text{cpm in B at } T_{10}\text{ s}}{\text{cpm in A at } T_{70}\text{ s} - \text{cpm in B at } T_{10}\text{ s}}\right) \times 100$$

Purine derivative	*mM*	*% Inhibition*
Hydroxynitrobenzylthioguanosine	0.025	100
Nitrobenzylthioinosine	0.0005	99
6-Benzyl-2-amino-9-β-D-ribofuranosylpurine	0.025	80
6-(Methylthio)-9-β-D-ribofuranosylpurine	5.0	95
6-Thioinosine	2.2	55
6-Thioguanosine	0.75	40
2′, 3′-*O*-Isopropylideneadenosine	3.2	90
2′-Deoxy-2′-thiomethyladenosine	2.2	85
3′-Deoxy-3′-methyladenosine	1.7	30
9-β-L-Ribofuranosyladenine	0.6	15
9-β-D-Psicofuranosyladenine	3.0	5

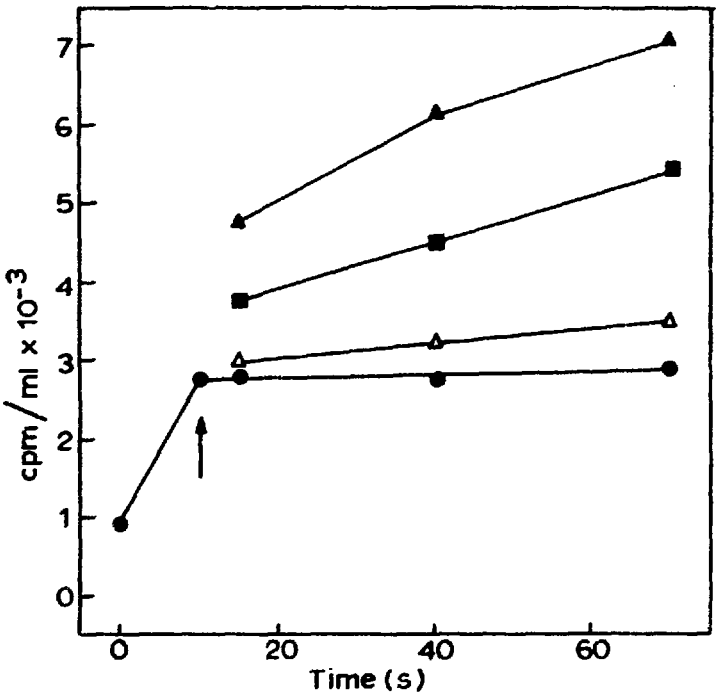

Fig. 4. Inhibition of uridine-accelerated efflux of uridine by 2′-deoxy-2′-thiomethyladenosine and 6-thioguanosine. At 0 s, efflux from cells loaded with 5.9 mM [2-^{14}C]uridine was initiated by addition of 10 volumes of buffered saline containing 5.9 mM non-radioactive uridine. At 10 s, additions were made (arrow) of 10 volumes of (1) buffered saline, (2) buffered saline containing hydroxynitrobenzylthioguanosine, or (3) buffered saline containing the test compound; final reaction concentrations are indicated below. At 15, 40, and 70 s, 1–2 ml portions were removed and centrifuged with dibutylphthalate, and radioactivity in cell-free supernatants was determined. ●, 0.025 mM hydroxynitrobenzylthioguanosine; △, 4.5 mM 2′-deoxy-2′-thiomethyladenosine; ■, 1.5 mM 6-thioguanosine; and ▲, buffered saline.

10 s (see calculation, Table V). The inhibitions reported in Table V were obtained by comparing the amount of radioactivity that appeared in the medium during 60 s of incubation in the presence of test compound with values obtained in the absence of test compound. Compounds were tested at concentrations similar to those used in experiments of Tables III and IV. The ribonucleosides with the greatest inhibitory activity (80–100% inhibition) were the *S*-substituted 6-thiopurine ribonucleosides and 6-benzyl-2-amino-9-β-D-ribofuranosylpurine. Of the adenine nucleosides with modifications in the sugar moiety, 2′,3′-*O*-isopropylidineadenosine had the greatest effect on efflux of uridine.

The range of concentrations of nitrobenzylthioinosine giving partial to complete inhibition of uridine-accelerated efflux of uridine are indicated in Table VI, where data from 1 of 3 similar experiments are presented. Cells loaded with 6 mM [2-^{14}C]uridine were incubated in medium containing (1) 6 mM uridine or (2) 6 mM uridine *plus* various concentrations of nitrobenzylthioinosine. Efflux of uridine was determined as described in Materials and Methods. Maximum inhibition of accelerative exchange diffusion of uridine was observed at nitrobenzylthioinosine concentrations of 1 μM and greater.

Data in Tables III and IV indicate inhibition of efflux by test nucleosides in the absence of extracellular uridine, whereas data in Tables V and VI are a measure of inhibition in the presence of extracellular uridine. Purine nucleosides

with inhibitory activity evidently interact with the uridine transport mechanism; however, as indicated in Table V, interaction of several of these compounds with the transport mechanism may be modified or prevented by the presence of extracellular uridine.

TABLE VI

INHIBITION OF URIDINE-ACCELERATED EFFLUX FROM URIDINE-LOADED ERYTHROCYTES BY NITROBENZYLTHIOINOSINE

% Inhibition is the rate of uridine outflow in the presence of inhibitor expressed as a percentage of the rate of outflow in the absence of inhibitor. Cells were loaded with 6.1 mM [2-^{14}C]uridine and reaction mixtures contained either 6.3 or 6.4 mM uridine.

μM	*μmoles/min per ml packed cells*	% *Inhibition*
0	5.29	0
0.01	4.81	9.1
0.025	3.71	23.6
0.05	3.57	26.2
0.1	1.72	67.5
0.15	0.41	91.5
0.25	0.48	90.1
0.4	0.33	93.8
1.0	0.11	97.9

DISCUSSION

Studies of monosaccharide transport in erythrocytes have shown that accelerative exchange diffusion occurs when structurally related permeants are present in the intracellular and extracellular compartments[14–17]. During accelerative exchange diffusion, the transport mechanism releases outgoing permeant at the external membrane face and reorients or returns to the internal membrane face more rapidly in the presence than in the absence of incoming permeant. The occurrence of accelerative exchange diffusion indicates that the accelerating and accelerated compounds involved are transported by the same mechanism of facilitated diffusion. Although kinetic models for exchange diffusion have been proposed[18–23], none satisfactorily explain all of the features of monosaccharide transport, and the molecular events underlying the process of exchange diffusion are unknown.

In the present work, accelerative exchange diffusion has been used to examine the specificity of the uridine transport mechanism in erythrocytes toward purine nucleosides by comparing the abilities of different purine nucleosides to enhance efflux of intracellular radioactive uridine. Acceleration of efflux by adenosine, inosine, guanosine, and arabinosyladenine indicates that the mechanism mediating the outward transport of uridine can also mediate transport of these purine nucleosides

Comparison of acceleration of efflux by purine nucleosides (Table I) with that by pyrimidine nucleosides (see ref. 3) suggests that fewer purine nucleosides

are accepted as permeants. Purine nucleosides accelerated efflux to a lesser extent than did pyrimidine nucleosides. When the extracellular medium contained adenosine, guanosine, inosine, or arabinosyladenine at concentrations from 4.0–6.9 mM, uridine fluxes were less than 71% of fluxes into medium containing uridine (Table I). Maximal acceleration of uridine efflux by thymidine, cytidine, and 5-amino-uridine were 92, 121 and 130%, respectively, or uridine-accelerated efflux[3].

These and earlier results[1–3] indicate that the nucleoside transport mechanism in human erythrocytes accepts (1) pyrimidine nucleosides with ribosyl, 2′-deoxyribosyl, or arabinosyl moieties, (2) purine nucleosides with ribosyl or arabinosyl moieties, and (3) certain C-glycosides (formycin B and pseudouridine). Compounds that had no effect on uridine efflux include free uracil, free sugars, and nucleotides; nucleosides with ionized groups on the base moiety and the 2′- and 3′-deoxyribosides of adenine also had no effect on efflux. It is apparent that modifications of the sugar moiety are poorly tolerated by the transport mechanism. Substitution on the 2′- or 3′-hydroxyl groups of uridine or cytidine greatly reduced permeant activity, and the 2′,3′-di-*O*-methyl and 2′,3′-*O*-isopropylidene derivatives of uridine inhibited uridine efflux[3]. Isopropylideneadenosine (Table V) appears to be a more effective inhibitor of uridine transport than isopropylideneuridine[3] because only the adenosine derivative prevented the acceleration of uridine efflux by 6 mM extracellular uridine.

Thioinosine and thioguanosine inhibited efflux of radioactive uridine into buffered saline (Table III) and into extracellular uridine (Table V); in contrast, 4-thiouridine was a substrate for the transport mechanism since it accelerated efflux of uridine[3]. Hydroxynitrobenzylthioguanosine was one of the most effective inhibitors of uridine transport; inhibition by the analogous *S*-substituted derivative of uridine, 4-(2-hydroxy-5-nitrobenzyl)thiouridine was complete only in the absence of extracellular uridine[3]. Comparison of the inhibitory effects of *S*-substituted and isopropylidene derivatives of purine ribonucleosides with those of pyrimidine ribonucleosides suggests that the transport inhibitors with purine moieties have a greater affinity for the transport mechanism than those with pyrimidine moieties.

It would appear that nucleosides are able to enter mammalian cells by means other than the uridine transport mechanism, because several of the purine nucleosides that inhibited or had no effect on uridine efflux are metabolized, as may be seen in the following examples. 2′-Deoxyadenosine was degraded by human erythrocytes with conversion of the 2′-deoxyribosyl moiety to lactic acid[23]. 9-β-D-Xylofuranosyladenine was rapidly deaminated by human blood cells in phosphate buffered medium[24], and 6-(methylmercapto)purine ribonucleoside accumulated as the 5′- monophosphate in human erythrocytes[25]. When Ehrlich ascites cells were incubated with 2′-deoxyadenosine, 2′-deoxy-ATP accumulated[26], and when these cells were incubated with 3′-deoxyadenosine, 3′-deoxy-ATP was found[27–29].

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REFERENCES

1 J. M. Oliver and A. R. P. Paterson, *Can. J. Biochem.*, 49 (1971) 262.
2 A. R. P. Paterson and J. M. Oliver, *Can. J. Biochem.*, 49 (1971) 271.
3 C. E. Cass and A. R. P. Patterson, *J. Biol. Chem.*, 247 (1972) 3314.
4 T. S. Lieu, R. A. Hudson, R. K. Brown and E. C. White, *Biochim. Biophys. Acta*, 241 (1971) 884.
5 P. G. W. Plagemann, *J. Cell. Physiol.*, 77 (1971) 213.
6 W. D. Stein, *The Movement of Molecules Across Cell Membranes*, Academic Press, New York 1967, p. 126.
7 D. Danon and Y. Marikovsky, *J. Lab. Clin. Med.*, 64 (1964) 668.
8 G. A. Bray, *Anal. Biochem.*, 1 (1960) 279.
9 G. W. Crabtree and J. F. Henderson, *Cancer Res.*, 31 (1971) 985.
10 S. V. Manohar, M. H. Lerner and D. Rubinstein, *Can. J. Biochem.*, 46 (1968) 445.
11 F. L. Meyskens and H. E. Williams, *Biochim. Biophys. Acta*, 240 (1971) 170.
12 B. Y. Kim, S. Cha and R. E. Parks, *J. Biol. Chem.*, 243 (1968) 1763.
13 R. P. Agarwal and R. E. Parks, *J. Biol. Chem.*, 246 (1971) 3763.
14 T. Rosenberg and W. Wilbrandt. *J. Gen. Physiol.*, 41 (1957) 289.
15 R. D. Mawe and H. G. Hempling, *J. Cell. Physiol.*, 66 (1965) 95.
16 M. Levine, D. L. Oxender and W. D. Stein, *Biochim. Biophys. Acta*, 109 (1965) 151.
17 M. Levine and W. D. Stein, *Biochim, Biophys. Acta*, 127 (1966) 179.
18 W. F. Widdas, *J. Physiol. London*, 118 (1952) 23.
19 P. G. Lefevre and G. F. McGinnis, *J. Gen. Physiol.*, 44 (1960) 87.
20 D. M. Miller, *Biophys. J.*, 8, (1968) 1329.
21 D. M. Miller, *Biophys. J.*, 8, (1968) 1339.
22 W. R. Lieb and W. D. Stein, *Biophys. J.*, 10 (1970) 585.
23 B. A. Lowy, E. R. Jaffe, G. A. Vanderhoof, L. Crook and I. M. London, *J. Biol. Chem.*, 230 (1958) 409.
24 R. Koshiura and G. A. LePage, *Cancer Res.*, 28 (1968) 1014.
25 T. L. Loo, D. H. W. Ho, D. R. Blossom, B. J. Shepard and E. Frei, *Biochem. Pharmacol.*, 18 (1969) 1711.
26 A. Coddington and M. Bagger-Sorensen, *Biochim. Biophys. Acta*, 72 (1963) 598.
27 H. Klenow, *Biochim. Biophys. Acta*, 76 (1963) 347.
28 H. Klenow and S. Frederikson, *Biochim. Biophys. Acta*, 87 (1964) 495.
29 H. Klenow and K. Overgaard-Hansen, *Biochim. Biophys. Acta*, 80 (1964) 500.
30 S. J. D. Karlish, W. R. Lieb, D. Ram and W. D. Stein, *Biochim. Biophys. Acta*, 255 (1972) 126.