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NUCLEOSIDE TRANSPORT IN SHEEP ERYTHROCYTES: GENETICALLY CONTROLLED TRANSPORT VARIATION AND ITS INFLUENCE ON ERYTHROCYTE ATP CONCENTRATIONS

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SUMMARY

1. The permeability of sheep erythrocytes to purine and pyrimidine nucleosides was investigated. Erythrocytes from most sheep (nucleoside-impermeable) were almost completely impermeable to 5 mM inosine whereas cells from approximately 5% of the animals studied (nucleoside-permeable) showed a rapid inosine uptake. Cells from both types of animal were permeable to 5 mM adenosine, although transport was slower in nucleoside-impermeable erythrocytes.

2. Two distinct nucleoside transport routes were present in nucleoside-permeable erythrocytes; a high affinity (apparent $K_m \simeq 0{\cdot}2$ mM) facilitated diffusion system which transported both purine and pyrimidine nucleosides, and a non-saturable uptake route selective for adenosine. The high affinity system was the major route of adenosine transport at physiological concentrations.

3. Transport by the high affinity system was completely inhibited by micromolar concentrations of dipyridamole and nitrobenzylthioinosine. Dipyridamole had no effect on the non-saturable component of adenosine uptake.

4. The transport differences between nucleoside-permeable and impermeable erythrocytes were due to the absence of the high affinity system from nucleoside-impermeable cells.

5. Nucleoside-permeable cells had a higher intracellular ATP concentration than nucleoside-impermeable erythrocytes, suggesting that the high affinity transport system participates in the energy metabolism of the cell.

INTRODUCTION

A number of investigations have demonstrated that nucleosides cross the erythrocyte membrane by facilitated diffusion, and studies of the human erythrocyte suggest the presence of a single broad specificity transport system for both purine and pyrimidine nucleosides (Whittam, 1960; Lieu, Hudson, Brown & White, 1971; Oliver & Paterson, 1971; Cass & Paterson, 1972; Pickard & Paterson, 1972*a*, *b*; Cass & Paterson, 1973). Bases and monosaccharides are not substrates for this system. Kinetic analyses (Cabantchik & Ginsburg, 1977) and inhibitor binding experiments (Pickard, Brown, Paul & Paterson, 1973; Cass, Gaudette & Paterson, 1974; Pickard & Paterson, 1976) have emphasized the usefulness of the erythrocyte in studies of the detailed mechanism of nucleoside translocation across cell membranes in general, but

there is little direct evidence concerning the physiological significance of nucleoside transport in these cells (see for example Brewer, 1974; Berlin & Oliver, 1975).

One experimental approach to the investigation of the mechanism of nucleoside transport and the clarification of its physiological significance is the study of genetically controlled membrane transport variants. McManus & Lambe (1972) reported that erythrocytes from some sheep synthesized ATP when incubated with adenosine, inosine and inorganic phosphate whereas cells from other sheep were unable to do so. Since erythrocytes from both types of animal were equally capable of synthesizing ATP when inosine was replaced by glucose, and since haemolysates from the two types of sheep metabolized inosine at the same rate, it was suggested that erythrocytes from these animals differed in their permeability to inosine (see Fig. 1 for a summary of the metabolic reactions involved). The object of the present study was to investigate the possibility of nucleoside transport variation in sheep erythrocytes. Evidence is presented which demonstrates that erythrocytes from some sheep possess a high affinity nucleoside transport system. In contrast, erythrocytes from other sheep lack this system, and the permeability difference between the two types of cell is under genetic control. A comparison of the intracellular ATP concentrations of these two types of erythrocyte suggests that this nucleoside transport system may play a significant role in the energy metabolism of the cell. Preliminary reports of some of these results have already appeared (Young, 1976, 1977).

METHODS

All animals were maintained at Babraham under standard husbandry conditions. Whole blood from adult sheep was collected by jugular venepuncture into heparinized evacuated tubes. Samples for ATP estimation were immediately chilled. Purine nucleoside phosphorylase assays and haematocrit and haemoglobin estimations were performed as previously described (Archer, 1965; Tucker & Young, 1976). The kinetic and metabolic experiments in this paper refer to purine nucleoside phosphorylase high-type sheep erythrocytes (Tucker & Young, 1976).

Materials

Uniformly labelled [^{14}C]nucleosides were obtained from The Radiochemical Centre, Amersham, Bucks, U.K. Other reagents were obtained as below: adenine, AMP, ATP, 9-β-D-arabinofuranosyl adenine, deoxyadenosine, deoxyinosine, guanosine, hypoxanthine, inosine, thymidine, uridine, 5,5′-dithiobis-(2-nitrobenzoate) (DTNB), diazenedicarboxylic acid bis-(*N*,*N*-dimethylamide) (diamide) and firefly tails from Sigma (London) Chemical Co. Ltd., Kingston-upon-Thames, Surrey, U.K.; adenosine from BDH Chemicals Ltd., Poole, Dorset, U.K.; dipyridamole (Persantin injection) from Boehringer Ingelheim Ltd., Bracknell, Berks., U.K. Nitrobenzylthioinosine (6-[(4-nitrobenzyl)thio]-9-β-D-ribofuranosylpurine) was a generous gift from Dr A. R. P. Paterson, University of Alberta Cancer Research Unit, Edmonton, Canada.

Nucleoside transport studies

Erythrocytes were washed 3 times with 20 vol. of a medium containing 140 mM-NaCl, 5 mM-KCl, 20 mM-Tris-HCl (pH 7·2 at 37 °C), 2 mM-$MgCl_2$, 0·1 mM-EDTA and 5 mM-glucose. The buffy coat was discarded. The erythrocyte: leukocyte ratio of the final cell preparation was never less than 1000:1.

Nucleoside uptake was measured at 37 °C by mixing 0·2 ml. pre-warmed washed erythrocytes (haematocrit approximately 20%) with 0·2 ml. pre-warmed medium containing the appropriate ^{14}C-labelled nucleoside (0·1–0·5 μC/μmole). At predetermined time intervals (typically 30 sec to 15 min) incubations were stopped by the addition of 1 ml. ice-cold medium, and the cells were rapidly washed 4 times with 1 ml. ice-cold medium using an Eppendorf 3200 microcentrifuge

(10 sec, 15000 *g*). The washed packed cells were lysed with 0·5 ml. 0·5% (v/v) Triton X-100 in water and 0·5 ml. 5% (w/v) trichloroacetic acid was added. The precipitate was removed by centrifugation (30 sec, 15000 *g*), and 0·9 ml. of the supernatant transferred to 7 ml. scintillation fluid (0·1 g 1,4-bis[2(5-phenyloxazolyl)]benzene (POPOP), 5 g 2,5-diphenyloxazole (PPO) and 480 ml. Triton X-100 in 1 l. toluene). Samples were counted for radioactivity in a Packard Tricarb scintillation counter with quench correction. The data in this paper refer to initial uptake

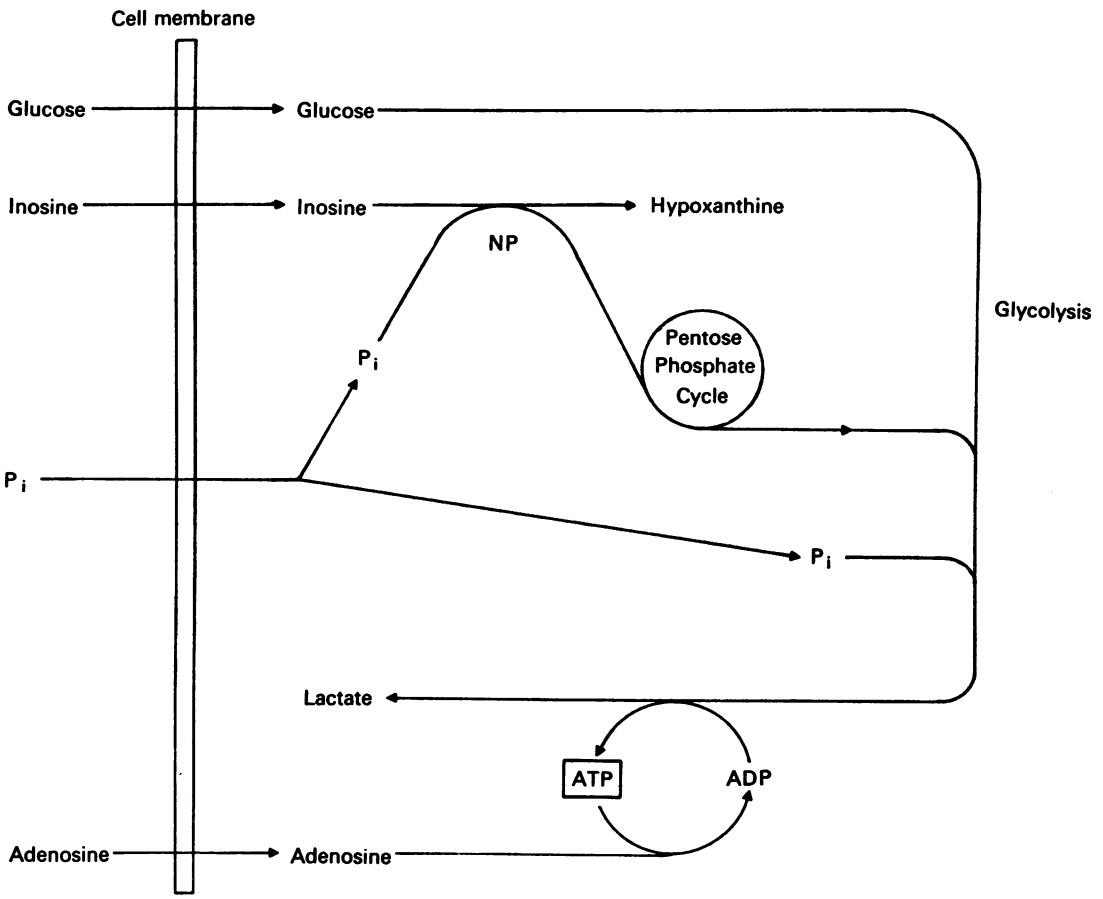

Fig. 1. Metabolic reactions involved in the synthesis of ATP from adenosine, phosphate, glucose and inosine in sheep erythrocytes. Inosine is metabolized via purine nucleoside phosphorylase (NP) (EC 2.4.2.1) to hypoxanthine and ribose-1-phosphate, which subsequently enters the pentose phosphate cycle as ribose-5- phosphate. Adenosine is initially phosphorylated by adenosine kinase (EC 2.7.1.20) to yield AMP which is converted to ADP by adenylate kinase (EC 2.7.4.3). Both adenosine kinase and adenylate kinase require ATP. Sheep erythrocytes do not have adenosine deaminase (EC 3.5.4.4) so that they cannot generate inosine from adenosine.

rates (maximum intracellular concentrations did not exceed 15% of extracellular concentrations). Control experiments established that the washing procedure did not result in loss of radioactivity from the cells. Similar uptake rates were found when extracellular isotope was removed by centrifuging the cells through a layer of *n*-dibutyl phthalate.

Metabolic studies

Erythrocyte ATP estimations. Whole blood ATP concentrations were estimated within 3 hr of bleeding by two independent methods: one a spectrophotometric NADH-enzyme assay (Boehringer Test Combination Diagnostics) and the other a fluorometric luciferin-luciferase assay (Beutler, 1971). Erythrocyte ATP concentrations were calculated using whole blood haematocrits. The ATP content of plasma and other cell elements is negligible (< 1%) (Beutler & Mathai, 1967).

In vitro incubation experiments. Erythrocytes were washed as described earlier except that the medium contained 140 mM-NaCl, 5 mM-KCl, 20 mM-sodium phosphate (pH 7·2) and 2 mM-$MgCl_2$. This medium was also used for the subsequent incubations.

The ability of intact erythrocytes to synthesize ATP from extracellular adenosine and phosphate in the presence of either glucose or inosine was measured by mixing 1·0 ml. ice-cold cell suspension (haematocrit approximately 40%) with 0·5 ml. ice-cold medium containing 15 mM-adenosine and either 15 mM glucose or inosine. A preincubation sample (0·25 ml.) was removed and immediately deproteinized with 1 ml. ice-cold 0·6 N-perchloric acid. The remaining cell suspension was incubated at 37 °C for 3 hr when a further 0·25 ml. aliquot was removed and deproteinized. ATP concentrations of the perchloric acid supernatants were determined by the spectrophotometric assay described above.

In other experiments the capacity of intact erythrocytes to utilize extracellular glucose or inosine to convert oxidized glutathione (GSSG) to the reduced form (GSH) was measured. Erythrocyte GSH was first oxidized by incubation of cells (haematocrit 20%) with 1 mM diamide (Kosower, Kosower, Wertheim & Correa, 1969) for 1 hr at 0 °C. The cells were then washed 4 times with 20 vol. ice-cold medium and resuspended to give a haematocrit of 20%. Erythrocyte suspensions (0·5 ml.) were incubated at 37 °C with an equal volume of medium containing either 10 mM glucose or inosine. At predetermined time intervals the cells were rapidly centrifuged at 15000 *g* for 10 sec. The cell pellet was lysed with 0·3 ml. ice-cold water and 0·6 ml. ice-cold 1·67% (w/v) glacial metaphosphoric acid containing 5 M-NaCl and 5·4 mM-EDTA added. The precipitate was removed by centrifugation (30 sec, 15000 *g*) and the GSH content of the supernatant estimated using the non-specific thiol reagent DTNB (Beutler, Duron & Kelly, 1963). Previous experiments have demonstrated that more than 92% of the DTNB-reactive thiol in sheep erythrocytes is GSH (Young, Nimmo & Hall, 1975).

RESULTS

Nucleoside permeability of sheep erythrocytes

In a preliminary series of experiments the initial rate of inosine uptake (5 mM) by erythrocytes from ninety-five sheep of the Finnish Landrace breed was determined. Table 1 summarizes the results obtained. Inosine uptake rates ranged from 0·02 to 2·9 m-mole/l. cells.hr and animals fell into two distinct groups on the basis of their permeability to the nucleoside. Erythrocytes from most animals were virtually impermeable to inosine, giving slow uptake rates in the range 0·02–0·18 m-mole/l. cells.hr. In contrast, cells from approximately 10% of the animals showed a rapid inosine uptake (2·2–2·9 m-mole/l. cells.hr). Typical time courses of inosine uptake by cells from the two types of animal are shown in Fig. 2. Sheep with erythrocytes showing a rapid inosine uptake were classified as inosine-permeable and the remainder as inosine-impermeable. Table 2 compares the frequency of inosine-permeability types in different sheep breeds. Of the 225 sheep tested, 5% were found to be inosine-permeable. The permeability characteristics of erythrocytes from individual animals remained constant during repeated analyses over a period of 2 years. Inosine-permeable animals were clinically normal, with no evidence of any haematological disorder.

In contrast to the situation with inosine, both inosine-permeable and impermeable

TABLE 1. Inosine uptake by erythrocytes from Finnish Landrace sheep

	Uptake (m-mole/l. cells per hr)	
	Inosine-permeable erythrocytes	Inosine-impermeable erythrocytes
Range	2·16–2·90	0·02–0·18
Mean ± S.E. of mean	2·44 ± 0·10	0·09 ± 0·005
No. of animals	8	87

The initial rate of inosine uptake (extracellular concentration 5mM) was determined as described in the text.

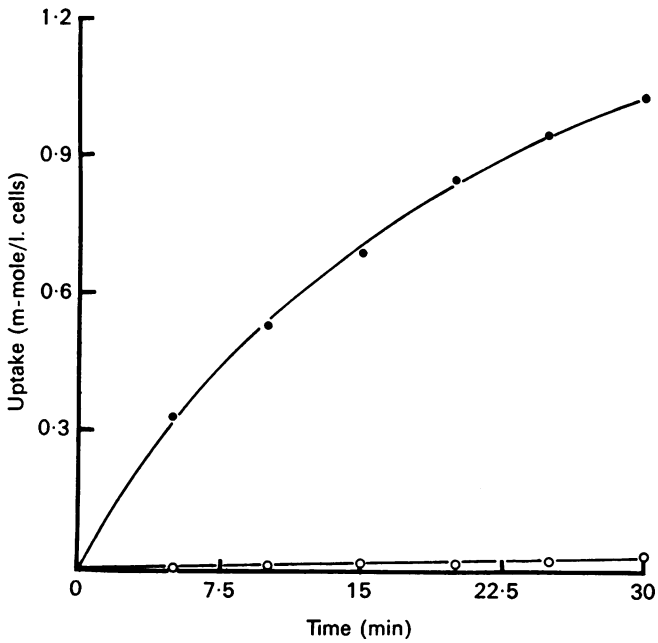

Fig. 2. Time course of inosine uptake by inosine-permeable and impermeable erythrocytes. The inosine concentration was 5 mM. ●, inosine-permeable cells; ○, inosine-impermeable cells.

TABLE 2. Frequency of inosine-permeability types in different sheep breeds

Breed	Inosine-permeable	Inosine-impermeable	Inosine-permeable (%)
Finnish Landrace	8	87	9·2
Finnish Landrace cross-bred	0	75	0
Clun Forest	2	51	3·9
Tasmanian Merino	1	1	—
Total	11	214	5·1

Animals were classified as to inosine-permeability type on the basis of erythrocyte inosine uptake rates (see text for details).

cells showed a rapid uptake of 5 mM adenosine. The permeability of inosine-impermeable cells was however significantly less than that of inosine-permeable erythrocytes ($2{\cdot}98 \pm 0{\cdot}10$[2·65–3·40] (7) and $1{\cdot}96 \pm 0{\cdot}03$[1·83–2·09] (6) m-mole/l. cells.hr for inosine-permeable and impermeable animals respectively (mean ± S.E. of mean [range] (n)), $P < 0{\cdot}001$).

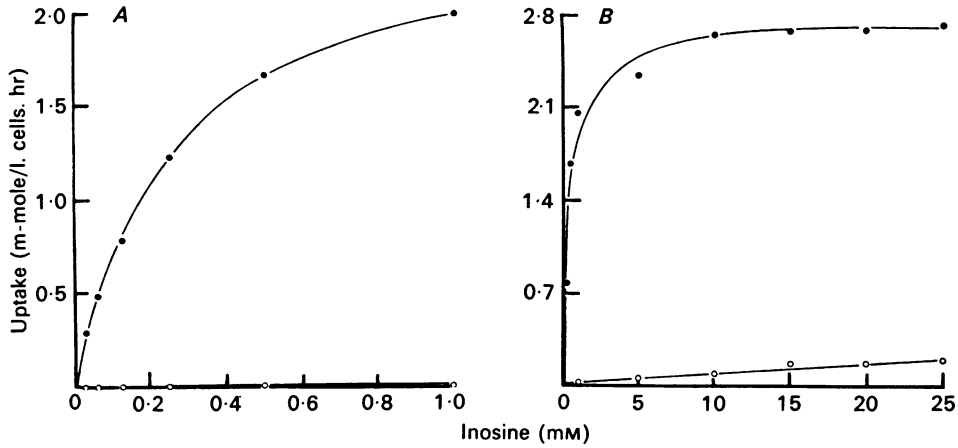

Fig. 3. Concentration dependence of inosine uptake by inosine-permeable and impermeable erythrocytes. The results of two experiments are shown: *A*, 0·03–1·0 mM; *B*, 0·1–25 mM. ●, inosine-permeable cells; ○, inosine-impermeable cells. The inosine-permeable curve in *A* is fitted as v (m-mole/l. cells·hr) $= 2{\cdot}50 \cdot s/0{\cdot}26 + s$, where s is the extracellular inosine concentration (mM).

Kinetics of nucleoside transport by inosine-permeable and impermeable erythrocytes

Concentration dependence of nucleoside transport. Fig. 3 shows the concentration dependence of inosine uptake by inosine-permeable and impermeable cells over the concentration range 0·03–25 mM. Uptake by inosine-permeable erythrocytes was saturable and conformed to simple Michaelis–Menten kinetics giving an apparent K_m of 0·26 mM and a V_{max} of 2·5 m-mole/l. cells.hr. In contrast, inosine-impermeable cells gave a slow linear uptake over the same concentration range (0·2 m-mole/l. cells.hr at 25 mM). Unlike purine nucleosides, pyrimidine nucleosides such as uridine and thymidine are not significantly cleaved or phosphorylated in human or other mammalian erythrocytes (Sandberg, Lee, Cartwright & Wintrobe, 1955; Oliver & Paterson, 1971). Fig. 4 shows the uptake of uridine (0·25–5 mM) by inosine-permeable and impermeable cells. As with inosine, uptake of uridine by inosine-permeable erythrocytes was rapid, and conformed to simple Michaelis–Menten kinetics (apparent K_m 0·47 mM, V_{max} 4·1 m-mole/l. cells.hr). Uridine uptake by inosine-impermeable cells was very slow and linear with concentration (0·06 m-mole/l. cells.hr at 5 mM). Fig. 5 shows the time course of uridine uptake (initial extracellular concentration 1·0 mM, haematocrit 10%) by inosine-permeable cells. A steady-state intracellular concentration of 0·60 m-mole/l. cells (extracellular concentration 0·92 mM) was achieved after 2 hr incubation, giving a distribution ratio (concentration of nucleoside in cell water: concentration in extracellular medium) of 0·86 (0·76 l. cell water.per l. cells).

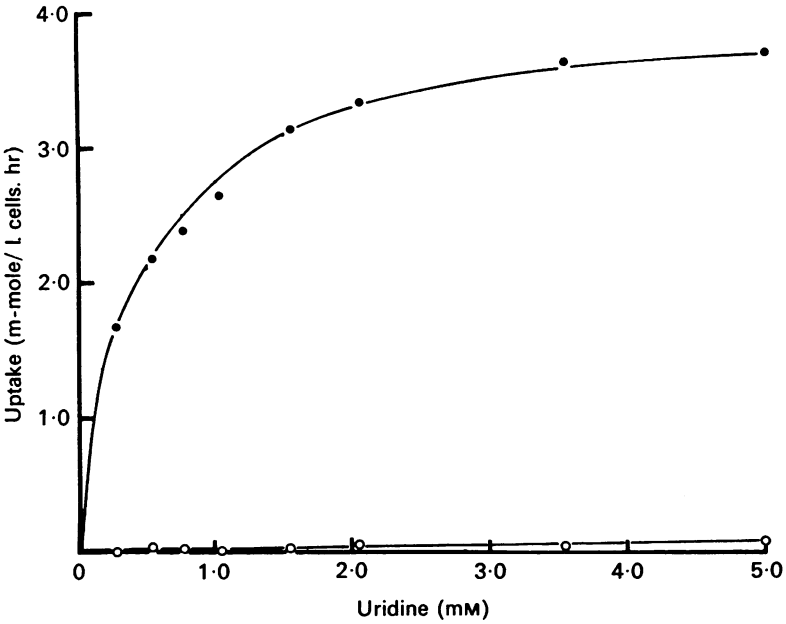

Fig. 4. Concentration dependence of uridine uptake by inosine-permeable and impermeable cells. ●, inosine-permeable cells; ○, inosine-impermeable cells. The inosine-permeable curve is fitted as v (m-mole/l. cells.hr) = $4{\cdot}10.\ s/0{\cdot}47+s$, where s is the extracellular uridine concentration (mM).

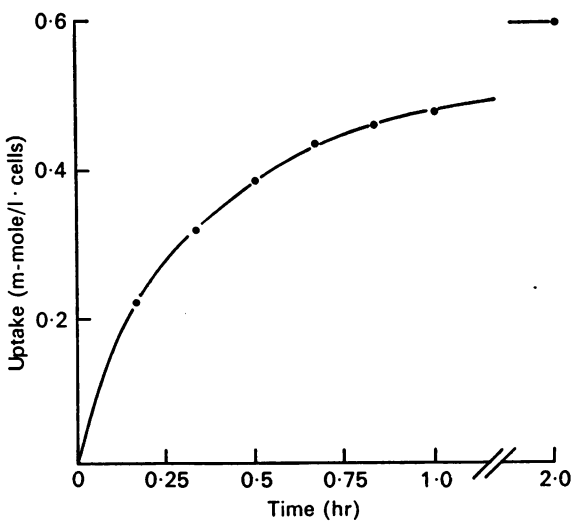

Fig. 5. Time course of uridine uptake by inosine-permeable cells. The initial extracellular uridine concentration was 1·0 mM. After 2 hr incubation the intracellular and extracellular uridine concentrations were 0·60 m-mole/l. cells and 0·92 mM respectively.

In contrast to the situation with inosine and uridine, the concentration dependence of adenosine uptake (0·03–25 mM) (Fig. 6) by inosine-permeable cells suggested the presence of two components of uptake: a saturable component (apparent K_m approximately 0·13 mM, V_{max} 1·4 m-mole/l. cells.hr) and a linear uptake component (4·7 m-mole/l. cells.hr at 25 mM). Only the non-saturable component of uptake was observed in inosine-impermeable erythrocytes (Fig. 6). The uptake of adenosine by inosine-impermeable cells was approximately 20-fold greater than that observed for either inosine or uridine.

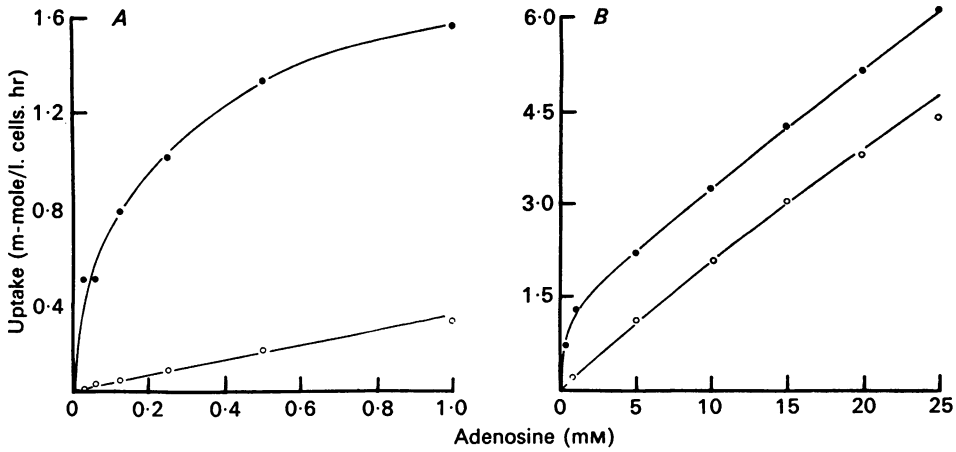

Fig. 6. Concentration dependence of adenosine uptake by inosine-permeable and impermeable erythrocytes. The results of two experiments are shown: *A*, 0·03–1·0 mM; *B*, 0·1–25 mM. ●, inosine-permeable cells; ○, inosine-impermeable cells.

A consequence of the adenosine concentration dependence curves in the two cell types is that at high adenosine concentrations the difference in uptake rate between the two cell types is relatively small. However, at low (physiological) adenosine concentrations the difference between the two cell types is considerable. In an experiment where the initial rate of adenosine uptake by inosine-permeable and impermeable cells was measured at an extracellular adenosine concentration of 30 μM, there was a 22-fold difference in uptake rate ($0{\cdot}344 \pm 0{\cdot}021$ and $0{\cdot}016 \pm 0{\cdot}001$ m-mole/l. cells.hr respectively (mean ± S.E. of mean (5)), $P < 0{\cdot}001$).

Competition studies. Fig. 7 shows the effect of 0·5–25 mM inosine on adenosine uptake (5 mM) by inosine-permeable and impermeable erythrocytes. Inosine inhibited adenosine uptake by inosine-permeable cells, but the inhibition was only partial even at high inosine concentrations (52% at 25 mM). In contrast, inosine had little effect on adenosine uptake by inosine-impermeable cells. The net effect of inosine was to abolish the adenosine permeability difference between the two cell types. Experiments performed at lower inosine and adenosine concentrations (0·05–1·0 mM) established that inosine was a simple competitive inhibitor of the saturable component of adenosine uptake in inosine-permeable cells (apparent K_i value 0·4 mM). Similarly adenosine was a competitive inhibitor of inosine uptake by inosine-permeable erythrocytes (apparent K_i 0·1 mM).

To assess the specificity of nucleoside transport by inosine-permeable cells a number of other nucleosides and bases were tested as inhibitors of inosine uptake. All the nucleosides tested were effective inhibitors of inosine influx with apparent K_i values in the range 0·1–0·5 mM (deoxyadenosine, 0·25 mM; 9-β-D-arabinofuranosyl adenine, 0·31 mM; deoxyinosine, 0·33 mM; guanosine, 0·33 mM; thymidine, 0·28 mM; uridine, 0·44 mM). There was no systematic discrimination between purine and pyrimidine nucleosides, but the differing K_i values for adenosine, deoxyadenosine and 9-β-D-arabinofuranosyl adenine suggest that the transport system may be sensitive

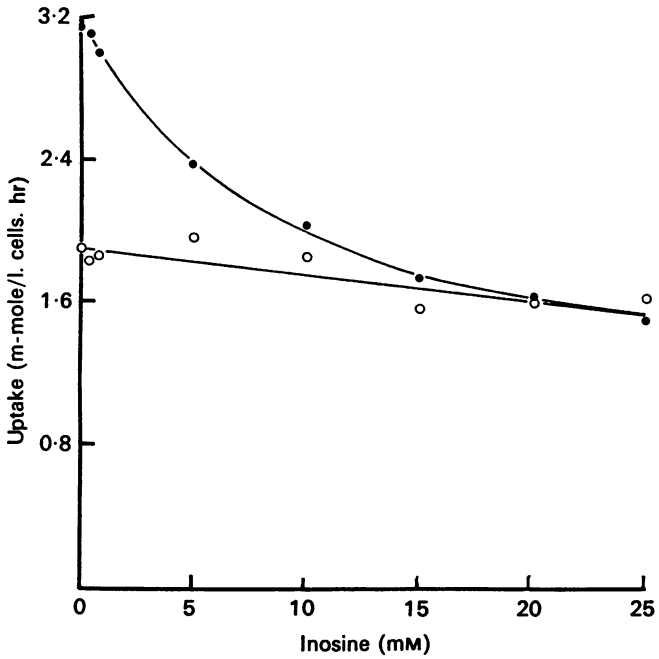

Fig. 7. Effect of inosine on adenosine uptake by inosine-permeable and impermeable erythrocytes. ●, inosine-permeable cells; ○, inosine-impermeable cells. See text for other experimental details.

to the carbohydrate part of the nucleoside. The apparent K_i values for adenosine and uridine as inhibitors of inosine uptake were almost identical to their apparent K_m values for the same system. Hypoxanthine, adenine and AMP did not significantly inhibit inosine transport.

Inhibitor studies. A number of vasodilator drugs and S-substituted 6-thiopurine ribonucleosides are potent inhibitors of nucleoside transport in a variety of cell types (see for example Berlin & Oliver, 1975). Dipyridamole and nitrobenzylthioinosine were both effective inhibitors of inosine uptake (1 mM) by inosine-permeable erythrocytes (> 85% inhibition at 1 μM).

Fig. 8 shows the effect of 20 μM dipyridamole on adenosine transport (0·1–7·5 mM) by both inosine-permeable and impermeable cells. Dipyridamole abolished the saturable component of adenosine transport in inosine-permeable erythrocytes, but had no effect on the non-saturable uptake route in either cell type.

Metabolic experiments

Erythrocyte ATP concentration

The intracellular ATP concentrations of inosine-permeable and impermeable sheep erythrocytes are given in Table 3. The concentration of ATP in inosine-permeable cells was significantly higher than that in inosine-impermeable erythrocytes, with both assay methods giving a value of 1·4 for the ratio (ATP concentration in permeable cells): (concentration in impermeable cells). In agreement with a previous study (Beutler & Mathai, 1967) the luciferin–luciferase assay gave significantly higher ATP levels than the spectrophotometric assay.

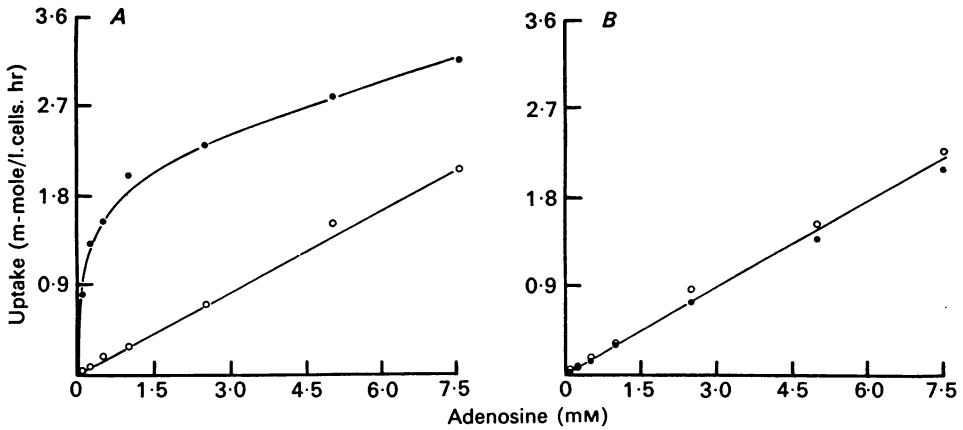

Fig. 8. Effect of dipyridamole on adenosine uptake by inosine-permeable and impermeable erythrocytes. *A*, inosine-permeable cells; *B*, inosine-impermeable cells. ●, control; ○, 20 μM dipyridamole. Adenosine and inhibitor were added to cell suspensions at the same time. Other experimental details are given in the text.

TABLE 3. ATP concentrations in inosine-permeable and impermeable erythrocytes

	ATP (m-mole/l. cells)			
	Inosine-permeable erythrocytes	Inosine-impermeable erythrocytes	Ratio (permeable: impermeable)	*P*
Luciferin–luciferase assay	1·52 ± 0·01	1·09 ± 0·07	1·39	< 0·01
NADH-enzyme assay	1·19 ± 0·04	0·88 ± 0·06	1·35	< 0·001
No. of animals	8	6		

Erythrocyte ATP concentrations (mean ± S.E. of mean) were estimated by two different methods as described in the Methods section. *P* values determined by Student's *t* test.

ATP synthesis

The ability of inosine-permeable and impermeable cells to synthesize ATP from extracellular adenosine and phosphate in the presence of either glucose or inosine is shown in Table 4. In these experiments adenosine, glucose and inosine were present at

a concentration of 5 mM and phosphate at 20 mM. Both cell types were equally capable of utilizing glucose for ATP synthesis. Inosine-permeable cells also synthesized considerable amounts of ATP when glucose was replaced by inosine. No ATP synthesis was observed however in inosine-impermeable cells under these conditions.

TABLE 4. ATP synthesis by inosine-permeable and impermeable erythrocytes

	Net ATP synthesis (m-mole/l. cells) (mean ± S.E. of mean)	
	Inosine-permeable erythrocytes	Inosine-impermeable erythrocytes
Adenosine (5 mM), glucose (5 mM) and phosphate (20 mM)	0·62 ± 0·11	0·53 ± 0·06
Adenosine (5 mM), inosine (5 mM) and phosphate (20 mM)	0·73 ± 0·13	0
No. of animals	3	3

Erythrocyte ATP was estimated by the NADH-enzyme assay. See text for other details.

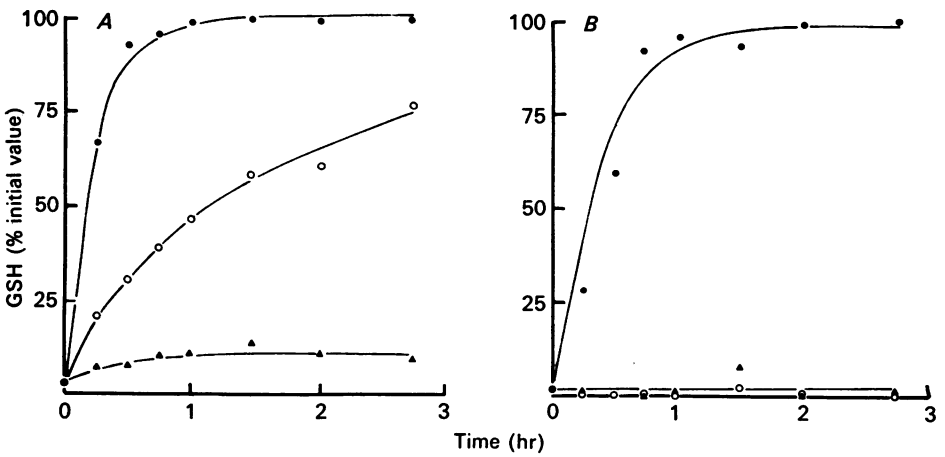

Fig. 9. GSSG reduction by inosine-permeable and impermeable erythrocytes. *A*, inosine-permeable cells; *B*, inosine-impermeable cells. Diamide-treated cells were incubated with medium containing 5 mM-glucose (●), 5 mM-inosine (○) or no additive (▲). Before diamide treatment the intracellular GSH concentrations of the inosine-permeable and impermeable cells were 2·13 and 2·85 m-mole/l. cells respectively.

GSH regeneration

Both inosine-permeable and impermeable erythrocytes could utilize glucose to regenerate GSH and GSSG after diamide oxidation (Fig. 9). The enzyme responsible for this conversion is the NADPH-dependent glutathione reductase (EC 1.6.4.2), and for both cell types regeneration was essentially complete after 1 hr incubation. Inosine-permeable cells were also able to use inosine as a source of NADPH. The rate of regeneration was slower than that observed with glucose, 75% regeneration occurring in 3 hr. In contrast, inosine-impermeable cells gave no inosine-dependent reduction of GSSG.

The considerable inosine-dependent reduction of GSSG by inosine-permeable cells eliminates the possibility that the rapid [^{14}C]nucleoside transport seen in these cell preparations reflects the properties of a small number of very active cells. Instead the experiments suggest that nucleoside permeability is a property of the whole cell population.

Nucleoside transport in other ruminant species

Table 5 summarizes the inosine permeability (5 mM) of goat, Barbary sheep (*Ammotragus lervia*), Mouflon (*Ovis musimon*) and cattle erythrocytes. The small number of animals from each of these species gave inosine uptake rates comparable to those of inosine-impermeable sheep cells.

TABLE 5. Nucleoside transport in other ruminant species

		Uptake (m-mole/l. cells.hr) (mean ± S.E. of mean (range))	No. of animals
Inosine* (5 mM)	Goat	0·057 ± 0·012 (0·044–0·081)	3
	Barbary sheep (*Ammotragus lervia*)	0·064 ± 0·005 (0·041–0·073)	11
	Mouflon (*Ovis musimon*)	0·037	1
	Cattle	0·111 ± 0·020 (0·079–0·147)	3

* For comparison with these data the mean inosine uptake rates (5 mM) for inosine-permeable and impermeable sheep erythrocytes were 2·44 and 0·09 m-mole/l. cells.hr respectively (Table 1).

DISCUSSION

The data presented in this paper demonstrate that sheep erythrocytes are divisible into two distinct types on the basis of their permeability to inosine. Cells from most animals (inosine-impermeable) were found to be virtually impermeable to the nucleoside whereas erythrocytes from approximately 5% of the animals studied showed a rapid inosine uptake (inosine-permeable). At an inosine concentration of 5 mM there was a 27-fold permeability difference between the two cell types. In contrast to the situation with inosine, both types of erythrocyte were permeable to adenosine at this concentration, although the uptake rate in inosine-permeable cells was significantly greater than that of inosine-impermeable erythrocytes.

Kinetic analyses revealed that inosine-permeable sheep erythrocytes possess two distinct nucleoside transport routes. The first is a high affinity (apparent $K_m \simeq 0{\cdot}2$ mM) facilitated diffusion system of broad specificity. This system does not discriminate between purine and pyrimidine nucleosides, but is sensitive to the carbohydrate component of the nucleoside. Hypoxanthine, adenine and AMP are not transported, and the system is completely inhibited by micromolar concentrations of dipyridamole and nitrobenzylthioinosine. The characteristics of this system are very similar to those of the nucleoside transport system in human erythrocytes, although human cells have a higher V_{max} (Oliver & Paterson, 1971; Cabantchik & Ginsburg, 1977). The second transport route is selective for adenosine and shows a linear concentration dependence. Transport by this route is not inhibited by dipyridamole and may represent simple diffusion through the lipid bilayer. The selectivity towards adenosine is probably a reflection of the relatively high lipid solubility of this nucleoside. Roos & Pfleger (1972) found that the octanol: water partition coefficient of

adenosine was elevenfold greater than that for inosine. The non-saturable uptake route predominates at high (> 5 mM) adenosine concentrations whereas the saturable uptake system is the major transport route at lower (i.e. physiological) adenosine concentrations. The results further demonstrate that the transport differences between inosine-permeable and impermeable cells are due to the absence of the high affinity transport system from inosine-impermeable erythrocytes. In view of the broad substrate specificity of this transport system it is more precise to describe the two cell types as nucleoside-permeable and nucleoside-impermeable. This nucleoside transport variation is independent of previously described amino acid and potassium transport variation in sheep erythrocytes (Young, Ellory & Tucker, 1976; Young & Ellory, 1977*a*, *b*; Ellory, 1977).

Inheritance data (J. D. Young, unpublished observations) suggest that this nucleoside transport variation is under genetic control, nucleoside-impermeability behaving as if dominant to nucleoside-permeability. The results are consistent with the involvement of two allelomorphic genes (Nu^i and Nu^I) where Nu^I codes for the absence of the high-affinity nucleoside transport system and is dominant to the gene coding for the presence of this system (Nu^i). Thus nucleoside-impermeable animals are either homozygous (Nu^I, Nu^I) or heterozygous (Nu^I, Nu^i) for the gene specifying nucleoside impermeability whereas nucleoside-permeable animals are homozygous (Nu^i, Nu^i) for the gene coding for nucleoside-permeability.

The presence of nucleoside transport variation in sheep erythrocytes allows the physiological significance of nucleoside transport in these cells to be assessed. Both nucleoside-permeable and impermeable erythrocytes synthesize ATP when incubated with high concentrations of adenosine, phosphate and glucose. Nucleoside-permeable but not impermeable cells can also utilize inosine in place of glucose. These cells therefore respond to extracellular adenosine and inosine in a similar way to the two cell types originally described by McManus & Lambe (1972). Other experiments demonstrate that inosine can replace glucose as a source of NADPH in nucleoside-permeable erythrocytes. These studies suggest the possibility that extracellular adenosine and inosine are physiological substrates for nucleoside-permeable sheep erythrocytes. It was therefore significant to find that nucleoside-permeable cells contained 40 % more ATP than nucleoside-impermeable erythrocytes, indicating that the high-affinity nucleoside transport system does participate in the energy metabolism of the cell *in vivo*.

Two distinct metabolic pathways could be responsible for the higher ATP concentration of nucleoside-permeable cells (see Fig. 1). First, adenosine may be utilized to increase the adenine nucleotide pool size. The nucleotides of mammalian erythrocytes undergo turnover as shown by both *in vitro* (Lowy, Ramot & London, 1960; Bishop, 1960) and *in vivo* studies (Lowy, Ramot & London, 1958; Bishop, 1961). These cells lack some of the enzymes required for *de novo* adenine biosynthesis, and both adenine and adenosine have been considered as possible precursors for adenine nucleotide synthesis (Meyskens & Williams, 1971; Brewer, 1974). A second possibility is that inosine may be acting as an energy source in addition to glucose. It may be possible to assess the relative significance of these two alternatives by investigating the influence of an inherited purine nucleoside phosphorylase deficiency which occurs independently in the erythrocytes of some sheep (Tucker & Young, 1976).

These results are also relevant to the identity of the physiological energy source of pig erythrocytes, which are impermeable to glucose (Kim & McManus, 1971*a*, *b*), but which rapidly transport nucleosides (J. D. Young, unpublished observations). It is therefore possible that nucleosides also contribute to the energy metabolism of these cells. Unlike sheep erythrocytes, erythrocytes from most pigs have a high adenosine deaminase activity so that they have the additional ability to convert adenosine to inosine.

In summary, it is concluded that erythrocytes from some sheep have a high-affinity nucleoside transport system which is absent from the erythrocytes of other sheep. Inheritance data indicate that the transport variation is under genetic control, and a comparison of the ATP concentrations of the two cell types suggests that the transport system participates in the energy metabolism of the erythrocyte. Further investigation of nucleoside-permeable and impermeable sheep erythrocytes may help elucidate the detailed mechanism of nucleoside transport across the erythrocyte membrane and further clarify its physiological significance.

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