Biochimica et Biophysica Acta, 778 (1984) 530–538
Elsevier

BBA 72389

UPTAKE OF URIC ACID, XANTHINE AND HYPOXANTHINE BY BRUSH-BORDER MEMBRANE VESICLES FROM MOUSE SMALL INTESTINE

MICHAEL I. SHAW and DENNIS S. PARSONS

Department of Biochemistry, South Parks Road, University of Oxford, Oxford OX1 3QU (U.K.)

(Received July 9th, 1984)

Key words: Brush-border membrane; Uric acid uptake; Xanthine uptake; Hypoxanthine uptake; (Mouse small intestine)

Using mouse small intestine brush-border membrane vesicles virtually free of xanthine oxidase (EC 1.2.3.2) and free of uricase (EC 1.7.3.3) the uptake of the purines uric acid, xanthine and hypoxanthine have been studied. The sodium-dependent overshoot phenomenon shown to exist for the uptake into the vesicles for D-glucose and L-phenylalanine was not observed with the purines. However, the uptake of the three purines in the presence of NaCl or KCl was greater than the uptake in the presence of either NaSCN or mannitol. Although 12.9% of the xanthine uptake and 17.6% of the hypoxanthine uptake was attributed to binding to the membranes, almost all the uric acid uptake was due to transport into an osmotically active space. The apparent intravesicular volume, calculated after 60 min incubation, for the three purines was consistently greater than the values obtained with D-glucose, L-glucose and L-phenylalanine equilibration, suggesting slow continuing penetration of purines associated with swelling or an apparent accumulation of purines within the vesicles associated with normal vesicle volume.

Introduction

The ability of the small intestine to transport sugars and amino acids against concentration gradients by specific sodium-dependent systems has long been established [1]. Similar proposals have also been made for the transport of the pyrimidines uracil [2–4] and thymine [5].

However, descriptions of the transport of the purines have been confusing: intestinal transport of uric acid has been reported as passive [6–8], as a 'probable' active secretion [9,10] and as occurring against an apparent concentration gradient [11,12] while that of xanthine has been reported as passive [7], as active secretion [9,10,13] and carrier mediated [14]. It has been suggested that hypoxanthine and/or its intestinal metabolites are absorbed by a saturable transport process [15], while the transport has been reported as passive [7] as active secretion [9,10,13], and Na^+ dependent [16].

When investigating the transport of purines by the small intestine a difficulty arises because hypoxanthine and xanthine are metabolised by the intracellular enzyme xanthine oxidase (EC 1.2.3.2) present in the mucosa [9]. Frequently allopurinol, an inhibitor of this enzyme [17], has been added in studies of purine transport but recent reports [12,13,18] suggest that allopurinol can also influence purine movements in the small intestine, so that interpretation of findings on purine transport in the presence of allopurinol is complicated. For these reasons we have investigated purine transport in a preparation of mouse small intestine brush-border membrane vesicles, virtually free of xanthine oxidase and describe here some observations on the uptake of uric acid, xanthine and hypoxanthine.

Materials and Methods

General. All reagents were of the highest purity available. Uric acid, hypoxanthine, xanthine, L-glucose, L-phenylalanine, phloridzin, ouabain, bovine serum albumin and tetrabutylammonium hydroxide were purchased from Sigma, Mannitol-AR, DNA and 4-nitrophenylphosphate from BDH, sucrose from East Anglia Chemicals and D-glucose from Fisons. Microporous filters (Amicon Ltd.) were used with an Amicon vacuum filtration manifold system (VFM1).

D-[U-^{14}C]Glucose, L-[1-^{14}C]glucose, L-[U-^{14}C]-phenylalanine, [2-^{14}C]uric acid, [6-^{14}C]xanthine and [8-^{14}C]hypoxanthine were all from Amersham International, U.K.

Animals. Adult male Balb-C mice, bred in the Department of Biochemistry, were kept in conditions of controlled temperature and daylight with free access to water and a stable laboratory diet (Dixons FFG (M) breeding diet).

Analytical procedures. Protein was determined by the method of Lowry et al. [19] using bovine serum albumin as reference, DNA by the method of Burton [20] using calf thymus DNA as reference. Alkaline phosphatase was determined by measuring the hydrolysis of 4-nitrophenylphosphate at pH 9.8 [21] and sucrase by measuring (by assay with hexokinase [22]) the glucose released from sucrose after incubation in 0.2 M phosphate buffer (pH 6.0).

Ouabain-sensitive ($Na^+ + K^+$)-ATPase activity was estimated by measuring the phosphate liberated [23] from ATP after incubation in a Tris (2-amino-2-hydroxymethylpropane-1,3-diol) buffer at pH 7.4 [24].

Purines were measured by high performance liquid chromatography (HPLC), with instrumentation and columns as previously described [12,25]. Two isocratic procedures were adopted:

(1) Ion-pair reverse phase chromatography with an Hypersil ODS column and 25 mM $NH_4H_2PO_4$ (pH 4.5), 6.25% (v/v) methanol, 2 mM tetrabutylammonium hydroxide as mobile phase, at a flow rate of 0.5 ml · min^{-1} and room temperature. Under these conditions of separation the retention times were allantoin 7.0, hypoxanthine 9.8, xanthine 11.2 and uric acid 20.7 min.

(2) Anion-exchange chromatography, using a Partisil 10 SAX column and 12.5 mM $NH_4H_2PO_4$ (pH 4.5) as mobile phase at a flow rate of 1.0 ml · min^{-1} and room temperature. Under this set of conditions allantoin elutes at the solvent front, uric acid 4.2, xanthine 5.0 and hypoxanthine 5.5 min. The metabolism, by brush-border membrane vesicles, of uric acid and xanthine were followed by method 1 and that of hypoxanthine by the anion-exchange method.

Radioactive isotope counting procedures were as previously described [12].

Electron microscopy. A portion of the brush border membrane vesicle pellet was fixed in 1% gluteraldehyde/ 2% formaldehyde in 0.1 M cacodylate buffer (pH 7.5), post fixed in osmium, dehydrated and embedded in resin. Thin sections were stained with uranyl acetate and lead citrate.

Preparation of brush-border membrane vesicles. The preparation of brush-border membrane vesicles was than of Kessler et al. [26] with slight modification. The animals were killed, the jejunum and ileum removed, flushed with ice-cold 0.9% (w/v) NaCl, opened along their length, blotted and the mucosa scraped off with a spatula. All further operations were carried out on ice or at 4°C. The scrapings were suspended in 50 mM mannitol/2 mM Tris-chloride buffer (pH 7.1) and homogenised in a Pulsematic 16 Osterizer (Oster Corp., U.S.A.) at maximum speed for 2 min. Solid $MgCl_2$ was added to the homogenate to give a final concentration of 10 mM. After standing for 15 min the suspension was centrifuged at 3000 × g for 15 min and the resulting pellet discarded. The supernatant was then centrifuged at 2700 × g for 30 min after first being filtered, if necessary, through a fine gauze to remove fatty material floating on the surface.

The resulting pellet was resuspended in 100 mM mannitol/10 mM Hepes (4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid)-Tris/0.1 mM $MgSO_4$ buffer (pH 7.5) and centrifuged at 30000 × g for a further 30 min. The pellet from this was resuspended in 300 mM mannitol/ 10 mM Hepes-Tris/0.1 $MgSO_4$ buffer (pH 7.5) to give a final protein concentration of 5-10 mg · ml^{-1} and used for the transport studies.

Purity of vesicle fraction. 2–3% of the homogenate protein and approx. 30% of the homogenate sucrase and of the alkaline phosphatase

activity appeared in the brush-border membrane vesicle fraction. The enrichment factors (vesicle/homogenate) were: sucrase 13.7 ± 0.6 (10), alkaline phosphatase 13.0 ± 0.7 (10), DNA (expressed as μg per mg protein) 0.10 ± 0.03 (7) and ouabain-sensitive $(Na^+ + K^+)$ATPase 1.1 ± 0.2 (7).

From electron microscopy the vesicle fraction contained vesicles 100–400 nm in diameter and was essentially free of all other organelles.

An extra slow spin ($3000 \times g$, 15 min) was initially included in our procedure between the 27 000 and the 30 000 $\times$ g spins, the resulting pellet from this slow spin being discarded. It was found that this pellet contained 7.1 ± 1.3 (3) % of the alkaline phosphatase and 7.0 ± 1.7 (3) % of the sucrase activity originally present in the homogenate so that the yield of the final brush-border membrane vesicle fraction was significantly decreased by the additional spin. Electron microscopy of this pellet formed from the slow spin showed it to be rich in vesicles, and when removing the supernatant it proved very difficult not to disturb the lightly packed pellet. For these reasons the extra step was not routinely included.

Transport studies. The uptake of substrates by brush border membrane vesicles was measured by a rapid filtration technique as described by Hopfer et al. [27]. Incubations were always performed on freshly prepared material at room temperature (21–23°C).

Aliquots of brush border membrane vesicles were incubated (1 : 1, v/v) with the incubation medium containing the substrate to be studied. The incubation media used were 10 mM Hepes-Tris/0.1 mM $MgSO_4$ (pH 7.5) containing 300 mM mannitol (medium 1), 100 mM NaCl/100 mM mannitol (medium 2), 100 mM NaSCN/100 mM mannitol (medium 3), 150 mM NaSCN (medium 4) and 100 mM KCl/100 mM mannitol (medium 5), ie. when medium 2 was mixed with brush-border membrane vesicles the final concentration of NaCl was 50 mM. At selected time intervals 50-μl aliquots of the reaction mixture were removed, applied directly to a filter and immediately washed with 2×3 ml of ice-cold stop solution of composition, 115 mM mannitol/100 mM NaCl/10 mM Hepes-Tris/0.1 mM $MgSO_4$ (pH 7.5). Corrections [26] for unspecific binding of radioactivity to the filters were made.

Brush-border membrane vesicles were incubated with D- and L-glucose at a specific activity of approx. $2 \cdot 10^4$ dpm/nmol and $1 \cdot 10^5$ dpm/nmol for the purines.

Microporous filters. Initially 0.45 μm (pore size) filters were used for the filtration step but it was found that 92.8 ± 1.5 (6) % of the protein and 80.6 ± 1.3 (6) % of the sucrase activity, present in the original sample applied to the filter, appeared in the filtrate.

When 0.2 μm filters were used 75.3 ± 2.2 (6) % of the protein and 57.1 ± 1.9 (6) % of the sucrase activity appeared in the filtrate. This was further illustrated by uptake studies with D-glucose. Incubations of brush-border membrane vesicles for 1 min with medium 4 and filtered through the 0.2 μm filters gave a value of 229.1 ± 12.3 (5) pmol/mg protein retained on the filter. Similar incubations but using 0.45 μM filters yielded only 48.3 ± 4.3 (5) pmol/mg protein. When two 0.45 μm filters were used, one on top of the other, and each counted separately the value obtained from the top filter was 68.2 ± 6.7 (5) and from the bottom filter 59.7 ± 5.9 (5) pmol/mg protein.

In all subsequent work the 0.2 μm pore size filters were used at the filtration step.

Uptake of D-glucose, uric acid, xanthine and hypoxanthine was directly proportional to the amount of protein applied to the filter.

Expression of results. Uptake by brush-border membrane vesicles is expressed as amount per unit protein, where the protein measured is that in the 50-μl aliquot removed from the reaction mixture and applied to the filter. Values are of means $\pm$ S.E. (for n determinations). Statistical comparisons are by Student's t-test.

Results

Glucose uptake

Fig. 1 shows the uptake into brush-border membrane vesicles from 0.1 mM D-glucose and demonstrates the electrogenic-dependent nature of the overshoot phenomenon and its dependence on the presence of Na^+ in the incubation medium. The uptake of D-glucose in the presence of 50 mM KCl (Table I) is markedly different from that obtained with either of the four other incubation media (Fig. 1). D-Glucose uptake was inversely

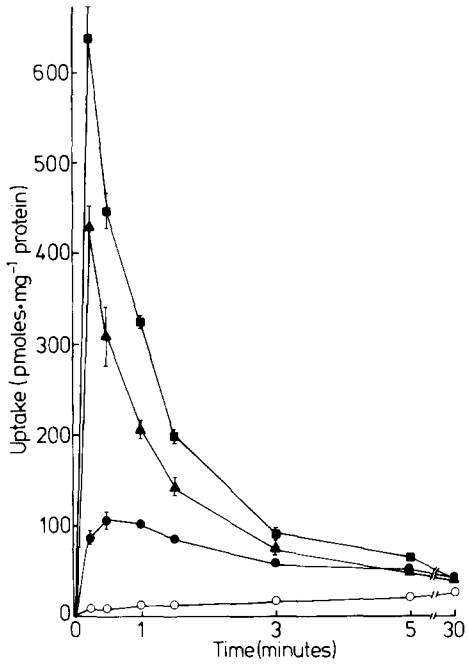

Fig. 1. Time-course of D-glucose (0.1 mM) uptake by mouse intestine brush-border membrane vesicles. Vesicles were suspended in 300 mM mannitol/10 mM Hepes-Tris/0.1 mM $MgSO_4$ (pH 7.5) and incubated 1:1 (v/v) with medium containing 10 mM Hepes-Tris/0.1 mM $MgSO_4$ (pH 7.5) and 300 mM mannitol (○), 100 mM NaCl/100 mM mannitol (●), 100 mM NaSCN/100 mM mannitol (▲), or 150 mM NaSCN (■). Each point is the mean ± S.E. of six determinations. (Where errors are not given, the points are larger.)

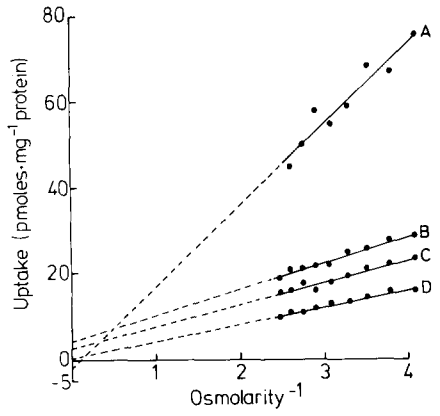

Fig. 2. Influence of osmolarity on D-glucose (0.1 mM) (A), hypoxanthine (12 μM) (B), xanthine (14 μM) (C) and uric acid (17 μM) (D) uptake by mouse intestine brush-border membrane vesicles. Vesicles were incubated for 5 min (glucose) or 10 min (purines) with medium containing 100 mM NaCl/10 mM Hepes-Tris/01. mM $MgSO_4$ (pH 7.5), mannitol to give the desired external osmolarity and the substrate to be followed. Each point is the mean of two determinations and the lines are fitted by linear-regression analysis.

proportional to the osmolarity of the incubation medium (Fig. 2), (slope, 19.1 ± 2.2 pmol/mg protein per osM; intercept, −2.23 ± 7.4; 6 degrees of freedom; P for significance of slope < 0.001). D-Glucose uptake was reduced by 83.9 ± 3.1 (6) % in the presence of 50 μM phloridzin and by 73.5 ± 2.2 (6) % at 0°C.

TABLE I

TIME-COURSE OF UPTAKE OF GLUCOSE AND PHENYLALANINE BY MOUSE SMALL INTESTINE BRUSH-BORDER MEMBRANE VESICLES

Vesicles were suspended in 300 mM mannitol/10 mM Hepes-Tris/0.1 mM $MgSO_4$ (pH 7.5) and incubated 1:1 (v/v) with medium containing 10 mM Hepes-Tris/0.1 mM $MgSO_4$ (pH 7.5) and 100 mM NaCl/100 mM mannitol (medium 2), 150 mM NaSCN (medium 4) or 100 mM KCl/100 mM mannitol (medium 5). Transport studies were carried out as in Materials and Methods. Values of uptake are pmol/mg protein ± S.E. for six determinations (L- and D-glucose) and three determinations (L-Phe).

Substrate and incubation medium	Time							
	15 s	30 s	60 s	90 s	3 min	5 min	30 min	60 min
L-Glucose (0.1 mM)								
Medium 4	9.2 ± 0.4	11.2 ± 0.9	14.8 ± 1.0	16.1 ± 1.6	22.5 ± 2.3	28.6 ± 4.5	44.5 ± 4.1	–
D-Glucose (0.1 mM)								
Medium 5	19.7 ± 3.6	31.2 ± 4.4	41.4 ± 6.6	67.5 ± 8.2	66.5 ± 6.1	70.7 ± 6.6	88.8 ± 7.3	62.5 ± 3.1
L-Phe (5.0 μM)								
Medium 2	2.6 ± 1.2	4.0 ± 1.0	6.8 ± 0.1	10.0 ± 1.8	6.4 ± 1.4	4.4 ± 1.4	3.4 ± 0.4	–

Assuming that complete equilibration of the substrate across the vesicle membrane has occurred after 60 min, the intravesicular volume can be calculated. For D-glucose the values are 0.39 ± 0.01 (6), 0.53 ± 0.03 (6), 0.57 ± 0.06 (6), 0.62 ± 0.01 (6) and 0.63 ± 0.03 (6) μl/mg protein with incubation media 1 (mannitol), 2 (NaCl), 3 (100 mM NaSCN), 4 (150 mM NaSCN) and 5 (KCl), respectively. There are no significant differences between the volumes obtained from incubations with media 2, 3, 4, and 5 (all $P > 0.1$), however, the intravesicular volume after incubation in the presence of 300 mM mannitol is significantly lower than the four other values (all $P < 0.02$).

Transport studies with 0.1 mM L-glucose showed no Na^+-dependent overshoot, and the uptake in the presence of 75 mM NaSCN is given in Table I. The findings for L-glucose uptake, over the same time-course, in the presence of 50 mM NaCl or 50 mM NaSCN were very similar, all comparisons $P > 0.1$ except for the 15 s values (6.4 ± 0.6 (6) pmol/mg protein with NaCl, $P < 0.01$, and 6.7 ± 0.4 (6) pmol/mg protein with 50 mM NaSCN, $P < 0.01$).

L-Phenylalanine

Table I also gives the uptake by brush-border membrane vesicles from 5 μM phenylalanine in the presence of 50 mM NaCl. An overshoot occurs but at a slightly later time than that found for D-glucose. The ratio of uptake at 90 s/30 min is 3.2 ± 0.8 (6).

Purine uptake

The time-course of the uptake by brush-border membrane vesicles, in different incubation conditions, from 17 μM uric acid are given in Table II, that from 14 μM xanthine in Table III and from 12 μM hypoxanthine in Table IV. There is no indication of a Na^+-dependent overshoot for the purines but it does seem that the uptake is dependent on the composition of the incubation buffer.

Uric acid

Uric acid uptake in the presence of 50 mM KCl is similar to that found with 50 mM NaCl, and the uptake with NaCl is greater than that observed from media containing NaSCN or 300 mM mannitol. For instance, in the case of uric acid the 15 s sample values for both KCl and NaCl incubations (Table II) are approximately double the mannitol and NaSCN values and remain consistently higher throughout the incubation period.

When the apparent intravesicular volume is calculated, assuming complete equilibration across the membranes at 60 min, values of 0.71 ± 0.01 (6), 1.09 ± 0.02 (6), 0.67 ± 0.05 (6), 0.64 ± 0.02 (6) and 0.98 ± 0.02 (6) μl/mg protein, for incubations with media 1 (mannitol), 2 (NaCl), 3 (100 mM NaSCN), 4 (150 mM NaSCN) and 5 (KCl), respectively are observed. The values found for incubations with NaCl or KCl present are significantly greater than those from 300 mM mannitol or NaSCN incubations (all $P < 0.001$). The value of 1.09 (NaCl present) is significantly greater ($P <$

TABLE II

TIME-COURSE OF URIC ACID (17 μM) UPTAKE BY MOUSE SMALL INTESTINE BRUSH-BORDER MEMBRANE VESICLES

Vesicles were suspended in 300 mM mannitol/10 mM Hepes-Tris/0.1 mM $MgSO_4$ (pH 7.5) and incubated 1:1 (v/v) with medium containing 10 mM Hepes-Tris/0.1 mM $MgSO_4$ (pH 7.5) and 300 mM mannitol (medium 1), 100 mM NaCl/100 mM mannitol (medium 2), 100 mM NaSCN/100 mM mannitol (medium 3), 150 mM NaSCN (medium 4), or 100 mM KCl/100 mM mannitol (medium 5). Transport studies were carried out as in Materials and Methods. Values of uptake are, pmol/mg protein ± S.E. for six determinations.

Incubation medium	Time							
	15 s	30 s	60 s	90 s	3 min	5 min	30 min	60 min
1	4.2±0.5	4.7±0.4	5.2±0.6	4.9±0.3	5.6±0.4	7.2±0.5	11.4±1.1	12.0±0.2
2	8.2±0.8	7.9±0.4	9.8±0.8	11.0±1.4	10.0±0.5	12.9±1.2	18.4±1.4	18.5±0.3
3	4.6±0.4	5.9±0.5	5.4±0.2	6.4±0.9	8.4±1.2	9.1±1.1	12.7±0.6	11.3±0.9
4	4.4±0.3	6.9±0.9	6.1±0.4	7.3±0.8	7.2±0.4	8.4±1.1	11.2±1.4	11.0±0.3
5	10.6±2.2	11.2±1.0	10.1±1.8	9.6±1.4	11.4±0.7	13.0±1.5	13.8±0.8	16.7±0.4

0.001) than the value of 0.98 (KCl present).

These values of apparent intravesicular volume obtained from incubation with uric acid, in the presence of NaCl, KCl or mannitol alone, are much higher than their respective values obtained from D-glucose equilibration. The data from the two NaSCN incubations are not significantly different ($P > 0.05$) from the respective values with D-glucose.

The uric acid present in the vesicles may be free in solution in the intravesicular space or bound to the membranes and associated structures. Fig. 2 shows that the uptake of uric acid is inversely related to the medium osmolarity, which was determined by different concentrations of mannitol. As the osmolarity of the medium approaches infinity, the intercept on the ordinate gives the uptake that is independent of intravesicular space i.e. binding. The line has a slope of 3.94 ± 0.3 pmol/mg protein per osM with an intercept of 0.42 ± 0.96 (7 degrees of freedom, $P < 0.001$) indicating that the uptake of uric acid under normal incubation conditions (300 mosM) is almost entirely due to transport since only 2.3% of the uptake at 300 mosM is due to binding.

Analysis of [2-^{14}C]uric acid by HPLC showed 98.0 ± 0.4 (4) % of the radioactive counts to be associated with the uric acid peak. After incubating with brush border membrane vesicles for 60 min 97.9 ± 0.2 (3) % of the counts were still associated with the uric acid peak, demonstrating that uric acid metabolism by brush border membrane vesicles proved negligible over the incubation period.

Xanthine

The uptake of xanthine (Table III) is consistently greater when NaCl or KCl is present in the incubation buffer. In the presence of NaCl from 90 s onwards and in the presence of KCl from 5 min onwards uptake is significantly greater (all $P < 0.02$) from the uptake with 300 mM mannitol or with NaSCN present in the incubation buffer.

The apparent intravesicular volumes calculated at 60 min from the xanthine data were 1.1 ± 0.09 (6), 1.76 ± 0.03 (6), 1.50 ± 0.07 (6), 1.54 ± 0.05 (6) and 1.73 ± 0.06 (6) μl/mg protein, for incubations with media 1 (mannitol), 2 (NaCl), 3 (100 mM NaSCN), 4 (150 mM NaSCN) and 5 (KCl), respectively. The values with NaCl and KCl present are not significantly different ($P > 0.1$) but both these values are significantly greater than the other three values ($P < 0.02$). The intravesicular volume obtained with incubation in 300 mM mannitol alone is significantly lower ($P < 0.01$) than the values from incubations in the other four conditions. These volumes are much larger than the respective volumes found with D-glucose.

Xanthine uptake is inversely related to the osmolarity of the medium (Fig. 2) with a slope of 5.16 ± 0.48 pmol/mg protein per osM and an intercept of 2.51 ± 1.52 (7 degrees of freedom, $P < 0.001$) indicating that 12.9% of the measured brush border membrane vesicle uptake at 300 mosM is due to binding. Even allowing for this value, the intravesicular volumes calculated from the xanthine uptake data are still greater than those from the D-glucose data.

TABLE III

TIME-COURSE OF XANTHINE (14 μM) UPTAKE BY MOUSE SMALL INTESTINE BRUSH-BORDER MEMBRANE VESICLES

For conditions of incubation see legend to Table II. Values of uptake are pmol/mg protein ± S.E. for six determinations.

Incubation medium	Time							
	15 s	30 s	60 s	90 s	3 min	5 min	30 min	60 min
1	8.2±1.6	9.8±2.2	12.3±3.1	10.0±2.1	13.9±1.0	15.0±1.1	16.7±1.0	16.0±1.4
2	8.1±3.0	10.8±1.6	13.0±2.3	15.8±1.2	18.4±2.0	18.2±0.9	21.2±1.6	24.6±0.5
3	6.3±1.7	6.7±1.3	8.1±1.0	9.0±1.7	10.7±1.2	12.5±1.3	15.7±1.1	21.0±1.0
4	4.9±0.9	6.8±1.0	8.7±1.2	9.6±1.2	7.4±1.6	11.0±1.1	14.1±1.7	21.6±0.7
5	6.3±1.5	6.9±1.5	11.4±1.8	11.3±1.6	14.4±1.9	19.7±1.8	21.2±1.3	24.2±0.8

TABLE IV

TIME-COURSE OF HYPOXANTHINE (12 μM) UPTAKE BY MOUSE SMALL INTESTINE BRUSH-BORDER MEMBRANE VESICLES

For conditions of incubation see legend to Table II. Values of uptake are pmol/mg protein ± S.E. for six determinations.

Incubation medium	Time							
	15 s	30 s	60 s	90 s	3 min	5 min	30 min	60 min
1	6.5 ± 1.1	10.4 ± 1.2	13.7 ± 2.7	15.0 ± 1.3	14.9 ± 2.1	21.9 ± 1.4	22.0 ± 0.9	19.9 ± 1.4
2	4.7 ± 0.5	10.2 ± 1.7	10.7 ± 1.7	10.8 ± 1.4	13.2 ± 1.6	24.3 ± 2.3	27.7 ± 2.4	25.9 ± 1.0
3	4.6 ± 1.6	8.3 ± 1.3	11.5 ± 1.3	8.9 ± 2.1	16.4 ± 1.1	20.3 ± 2.0	21.7 ± 1.6	18.6 ± 1.1
4	8.7 ± 0.9	9.9 ± 1.1	13.6 ± 0.9	11.3 ± 2.0	16.9 ± 1.8	23.0 ± 1.0	21.8 ± 1.5	16.7 ± 0.7
5	7.9 ± 1.3	7.0 ± 1.0	11.9 ± 2.1	13.1 ± 1.4	15.4 ± 1.4	22.1 ± 1.4	34.4 ± 3.3	26.3 ± 2.1

Analysis of [^{14}C]xanthine by HPLC showed 98.7 ± 0.2 (4) % of the counts to be associated with xanthine. After incubation with brush-border membrane vesicles for 10 min this value was 96.5 ± 0.3 (3) with 1.6 ± 0.2 (3) % of the counts associated with uric acid. After 30 min incubation 94.0% and 3.9% of the counts (mean of two incubations) were associated with xanthine and uric acid, respectively. After 60 min 91.6 ± 1.1 (3) % of the counts were associated with xanthine and 6.6 ± 0.9 (3) % with uric acid.

Hypoxanthine

Brush-border membrane vesicle uptake of hypoxanthine (Table IV) also shows a dependence on the medium composition, the greatest uptake again being found in the presence of NaCl and KCl. The differences in uptake are less marked than with uric acid and xanthine and are only really apparent after 30 min.

The apparent intravesicular volumes calculated from 60 min incubations gives 1.65 ± 0.1 (6), 2.15 ± 0.1 (6), 1.54 ± 0.1 (6), 1.39 ± 0.1 (6) and 2.19 ± 0.2 (6) μl/mg protein for incubations with media 1 (mannitol), 2 (NaCl), 3 (100 mM NaSCN), 4 (150 mM NaSCN) and 5 (KCl), respectively. The values from incubations with NaCl and KCl are significantly greater ($P < 0.02$) than those values from the other three incubation conditions and are much larger than the apparent volumes found with D-glucose. The uptake of hypoxanthine is inversely proportional to the osmolarity of the medium (Fig. 2) with a slope of 6.08 ± 0.37 pmol/mg protein per osM and an intercept of 4.31 ± 1.18 (7 degrees of freedom, $P < 0.001$), indicating that 17.6% of uptake at 300 mosM is binding. Allowing for this binding does not sufficiently decrease the intravesicular volume for it to be the same as for the values obtained for D-glucose equilibration.

Analysis of [^{14}C]hypoxanthine by HPLC showed 98.8 ± 0.1 (4) % of the counts to be associated with hypoxanthine. After incubation for 10 min with brush-border membrane vesicles 97.4 ± 0.3 (4) %, 1.4 ± 0.1 (4) % and 0.5 ± 0.05 (4) % of the counts were associated with hypoxanthine, xanthine and uric acid, respectively. After 30 min incubation the values were 96.2, 3.0 and 0.6% (each the mean of two values) and after 60 min incubation, 94.0 ± 0.3 (3), 4.4 ± 0.1 (3) and 1.2 ± 0.03 (3) %, respectively.

Discussion

We have shown that our preparation of mouse small intestine brush-border membrane vesicles possesses systems for the 'active' uphill transport of D-glucose [28] and L-phenylalanine [29]. In the case of D-glucose the transport is electrogenically dependent upon sodium, temperature dependent, markedly inhibited by phloridzin and appears to occur with negligible binding to the membranes into a space whose volume is inversely proportional to the osmolarity of the medium. With L-glucose no overshoot and no dependence upon sodium is observed.

Allowing for the finding in the present study that 75% of the protein applied to the filter appears in the filtrate, the intravesicular volume for D-glucose at equilibrium ranges from 1.56, in the presence of 300 mM mannitol, to 2.52 μl/mg

protein, in the presence of 50 mM KCl. These values are in accord with reported values for mouse brush-border membrane vesicles where 30 min equilibration with L-phenylalanine gave intravesicular volumes of between 1 and 2 μl/mg protein [29,30]. The intravesicular volumes calculated after 30 min incubation with L-phenylalanine and L-glucose (Table I) are 2.72 and 1.78 μl/mg protein, respectively. The fact that the value for D-glucose calculated after 60 min equilibration with medium 1 (300 mM mannitol) is lower than the values calculated after equilibration in the presence of the salts suggests that D-glucose has not fully equilibrated across the membranes after 60 min in the mannitol medium.

That the 0.2 μm Amicon filters retain only 25% of the protein applied shows that these filters behave differently from those used in previous studies (e.g., Millipore filters) where it has been suggested that the vesicles bind to the filter matrix [31] to the extent that almost all the protein applied to the filters is retained.

The fact that uric acid, xanthine and hypoxanthine are scarcely metabolised by the membranes demonstrates that the preparation is virtually free of xanthine oxidase and free of uricase, so that it is the uptake of the unmetabolised purines that is followed. We have shown that, at the concentrations used, uric acid, xanthine and hypoxanthine uptake do not exhibit a Na^+-dependent overshoot.

The intravesicular volume calculated for the purines are significantly greater than those found for D-glucose. Because uptake of uric acid by the vesicles has been shown to be due almost entirely to transport and not binding, either the volume of the vesicles has increased considerably and/or uric acid is accumulated during incubations in the presence of NaCl, KCl or 300 mM mannitol.

Similar results were obtained from incubations with xanthine and hypoxanthine and even allowing for the uptake which is due to binding (12.9 and 17.6%, respectively) and substrate metabolism (7.1 and 4.8%, respectively) the intravesicular volumes after 60 min incubations are still significantly above those calculated for D-glucose, L-glucose and L-phenylalanine.

The data presented also suggest that purine uptake is influenced by the composition of the incubation medium, the greatest uptake being found when either NaCl or KCl is present. It has been reported [29] that both Na^+ and K^+ gradients can elicit 'active' transport of L-phenylalanine in mouse brush-border membrane vesicles but that the influence of anions differs from that found for D-glucose: for example in the presence of a Na^+ gradient, the amino acid was transported more rapidly in the presence of Cl^- than SCN^-. In the present study the uptake of uric acid and xanthine is less in the presence of NaSCN compared with that for KCl or NaCl, but this is only noticeable for the uptake of hypoxanthine after 30 min.

In the presence of KCl the uptake of D-glucose (Table I) shows a much slower overshoot, when compared to the data in Fig. 1, almost as if a secondary gradient is being established as a consequence of the imposed KCl gradient (outside > inside). We can assume that the only driving forces provided are those derived from the salt gradients employed; i.e. any effect must come directly or indirectly from this imposed (out > in) salt gradient.

If the transport of the purines and the anomaly of KCl stimulated D-glucose overshoot are to be explained by an increase in intravesicular volume, i.e., swelling due to fluid movement, then a problem still remains. In the case of D-glucose there must be an initial swelling followed by a shrinkage because, in the presence of KCl, the uptake after 60 min is not significantly different from values obtained after incubations for 60 min in the presence of NaCl and NaSCN. These findings with the purines where the uptake remains high throughout the incubation period, suggest perhaps a slow continuing transport of purines associated with swelling. That KCl has such an affect on the D-glucose uptake and elicits a high uptake with the purines might imply direct stimulation by the presence of K^+; similar proposals have been made for glutamate in the kidney [32] and phenylalanine in the small intestine [30].

If in fact the purines are accumulated inside the vesicles this could be explained by specific transport systems in the membranes and a driving force, i.e. an electrochemical potential gradient across the membrane (e.g. a proton gradient, inside more alkaline due to Na^+-proton exchange). Alternatively it has been suggested that weak elec-

trolytes cross membranes by nonionic diffusion [33,34]. Such transport would obviously be governed by the pH of the two compartments adjacent to the membrane, implying that any favourable pH gradient established across the membrane of the vesicles could induce accumulation. The surprising observation in the present study is that the apparent accumulation of the purines also occurs when brush border membrane vesicles are incubated in buffer containing no salts but 300 mM mannitol, this could be explained if binding occurs when the ionic strength is very low, i.e. with mannitol only.

If specific transport systems exist for the purines, we must conclude that the uptake is so slow, compared with that of e.g. D-glucose that it is impossible to observe an overshoot.

Acknowledgements

This work was supported by MRC Project Grant G810 3574. We thank Mrs. Denise Mulvey for the DNA assays and J.E. Pearson for the electron microscopy.

References

1 Schultz, S.G. and Curran, P.F. (1970) Physiol. Rev. 50, 637–718
2 Schanker, L.S. and Tocco, D.J. (1962) Biochim. Biophys. Acta 56, 469–473
3 Czaky, T.Z. (1961) Am. J. Physiol. 201, 999–1001
4 Scharrer, E. and Amann, B. (1979) Ann. Rech. Vet. 10, 467–469
5 Schanker, L.S. and Tocco, D.C. (1960) J. Pharmacol. Exptl. Ther. 128, 115–121
6 Oh, J.H., Dossetor, J.B. and Beck, I.T. (1967) Can. J. Physiol. Pharmacol. 45, 121–127
7 Khan, A.H., Wilson, S. and Crawhall, J.C. (1975) Can. J. Physiol. Pharmacol. 53, 113–119
8 Dukes, C.E., Steplock, D.A., Kahn, A.M. and Weinman, E.J. (1982) Proc. Soc. Exptl. Biol. Med. 171, 19–23
9 Berlin, R.D. and Hawkins, R.A. (1968) Am. J. Physiol. 215, 932–941
10 Berlin, R.D. and Hawkins, R.A. (1968) Am. J. Physiol. 215, 942–950
11 Parsons, D.S. and Shaw, M.I. (1983) Q. J Exptl. Physiol. 68, 53–67
12 Shaw, M.I. and Parsons, D.S. (1984) Clin. Sci. 66, 257–268
13 Kolassa, N., Schutzenberger, W.G., Wiener, H. and Turnheim, K. (1980) Am. J. Physiol. 238, G141–149
14 Taube, R.A. and Berlin, R.D. (1970) Am. J. Physiol. 219, 666–671
15 Schanker, L.S., Jeffrey, J.J. and Tocco, D.J. (1963) Biochem. Pharmacol. 12, 1047–1053
16 Scharrer, E., Raab, B., Tiemeyer, W. and Amann, B. (1981) Pflugers Arch. 391, 41–43
17 Spector, T. (1977) Biochem. Pharmacol. 26, 353–358
18 Simmonds, H.A., Rising, T.J., Cadenhead, A., Hatfield, P.J., Jones, A.S. and Cameron, J.S. (1973) Biochem. Pharmacol. 22, 2553–2563
19 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
20 Burton, K. (1956) Biochem. J. 62, 315–323
21 Klaus, W. and Schutt, C. (1971) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.), Vol. 2, pp. 856–860
22 Bergmeyer, H.U., Bernt, E., Schmidt, F. and Stork, H. (1971) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.), Vol. 3, pp. 1196–1201
23 Sumner, J.B. (1944) Science 100, 413–414
24 Klodes, I., Ottolenghi, P. and Boldyrev, A. (1975) Anal. Biochem. 67, 397–403
25 Parsons, D.S. and Shaw, M.I. (1983) Q. J. Exptl. Physiol. 68, 39–51
26 Kessler, M., Acuto, O., Storelli, C., Murer, H., Muller, M. and Semenza, G. (1978) Biochim. Biophys. Acta 506, 136–154
27 Hopfer, U., Nelson, K., Perotto, J. and Isselbacher, K.J. (1973) J. Biol. Chem. 248, 25–32
28 Murer, H. and Hopfer, U. (1974) Proc. Natl. Acad. Sci. USA 71, 484–488
29 Berteloot, A., Khan, A.H. and Ramaswamy, K. (1982) Biochim. Biophys. Acta 691, 321–331
30 Berteloot, A., Khan, A.H. and Ramaswamy, K. (1981) Biochim. Biophys. Acta 649, 179–188
31 Ruzycki, S.M., Kelley, L.K. and Smith, C.H. (1978) Am. J. Physiol. 234, C27–35
32 Burckhardt, G., Kinne, R., Strange, G. and Murer, H. (1980) Biochim. Biophys. Acta 599, 191–201
33 Schanker, L.S., Dominick, J.T., Brodie, B.B. and Hogben, C.A.M. (1958) J. Pharmacol. Exptl. Ther. 123, 81–88
34 Hogben, C.A.M., Tocco, D.J., Brodie, B.B. and Schanker, L.S. (1959) J. Pharmacol. Exptl. Ther. 125, 275–282