Biochimica et Biophysica Acta, 597 (1980) 112—124

BBA 78635

SUGAR UPTAKE BY INTESTINAL BASOLATERAL MEMBRANE VESICLES

ERNEST M. WRIGHT, CAREL H. VAN OS * and AUSTIN K. MIRCHEFF **

Department of Physiology, University of California Medical Center, Los Angeles, CA 90024 (U.S.A.)

(Received April 27th, 1979)
(Revised manuscript received July 24th, 1979)

Key words: Glucose transport; Na^+ gradient; Facilitated diffusion; Sugar transport; (Intestinal epithelium)

Summary

A high yield of membrane vesicles was prepared from the basolateral surface of rat intestinal cells using an N_2 cavitation bomb and density gradient centrifugation. The membranes were enriched 10-fold and were free of significant contamination by brush border membranes and mitochondria. The rate of D-[^{14}C]-glucose and L-[^{3}H]glucose uptake into the vesicles was measured using a rapid filtration technique. D-Glucose equilibrated within the vesicles with a half-time 1/25th that for L-glucose. The stereospecific uptake exhibited saturation kinetics with a K_m of approx. 44 mM and a V of approx. 110 nmol · mg^{-1} · min^{-1} at 10°C. The activation energy for the process was 14 kcal · mol^{-1} below 15°C and it approached 3 kcal · mol^{-1} above 22°C. Carrier-mediated uptake was eliminated in the presence of 1 mM $HgCl_2$ and 0.5 mM phloretin. The rate of transport was unaffected by the absence or presence of sodium concentration gradients. Competition studies demonstrated that all sugars with the D-glucose pyranose ring chair conformation shared the transport system, and that, with the possible exception of the -OH group at carbon No. 1, there were no specific requirements for an equatorial -OH group at any position in the pyranose ring. In the case of α-methyl-D-glucoside its inability to share the D-glucose transport system may be due to steric hindrance posed by the -OCH_3 group rather than by a specific requirement for a free hydroxyl group at this position in the ring. It is concluded that sugars

* Present address: Department of Physiology, University of Nijmegen, Nijmegen, The Netherlands.
** Present address: Department of Physiology and Biophysics, USC Medical School, Los Angeles, CA 90033, U.S.A.
Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

are transported across the basolateral membrane of the intestinal epithelium by a facilitated diffusion system reminiscent of that in human red blood cells.

Introduction

Sugars are transported across the small intestine by a two stage process: the first is the entry of sugar into the epithelium across the brush border membrane and the second is the efflux of sugar out of the cell across the basolateral membrane into the blood. The transport mechanisms in the brush border membranes have been well characterized through the use of flux measurements in both intact tissues [1] and isolated brush border membranes [2]. It is concluded that the major transport mechanism in the brush border membrane is the so-called sodium co-transport system, where the sugar is transported across the membrane against its chemical potential gradient through a coupling to the sodium electrochemical potential gradient across the membrane.

Sugar transport across the basolateral plasma membrane has received little attention due to the inaccessibility of the serosal pole of the epithelium cell. Bihler and Cybulsky [3] blocked sugar transport across the brush border membrane by perfusing the gut lumen with $HgCl_2$ and then studied the uptake of sugars into isolated epithelial cells: they concluded that under these circumstances sugars entered, presumably across the basolateral membrane, by a facilitated transport system. Similar experiments on isolated cells were carried out by Kimmich and coworkers [4–6] and they concluded that the sodium-independent transport of 2-deoxy-D-glucose and 3-*O*-methyl-D-glucose into the cells occurred across the basolateral membranes by facilitated diffusion. Preliminary reports have appeared on the use of isolated basolateral membrane vesicles to study sugar transport [7,8] and these too suggest that sugars are transported by a sodium-independent facilitated diffusion mechanism.

Recently, we have developed procedures for the isolation of pure basolateral membranes from the rat intestinal epithelium [9–13], and we now have commenced a study of their transport properties. As a starting point for the characterization of our membranes, we have set out to confirm and extend the preliminary observations made by Murer and Hopfer [2] on the mechanism of sugar transport. We have established that these membranes exhibit a stereospecific, sodium-independent sugar transport system very similar to that found in human red blood cells.

Methods

Basolateral membranes were prepared from epithelial cells isolated from the rat duodenum, mid-jejunum or ileum, which were obtained by the method of Stern [14]. The isolated cells were harvested by centrifugation at $450 \times g$ for 5 min and were homogenized by a nitrogen cavitation technique similar to that first introduced to the intestine by Douglas et al. [15]. Cells (1–3 g wet weight) were suspended in 10 ml of buffer containing 25 mM NaCl and 1 mM Tris-HCl at pH 8 (bomb buffer) and were placed in a Kontes (Vineland, NJ) minibomb. We found that equilibration of the cells for 30 min at 1000 lb/

inch^2 (6900 kN/m^2) provided a convenient force to rupture greater than 90% of the cells. After rapid decompression, the homogenate was diluted 6-fold with bomb buffer (we found it unnecessary to add EDTA to the homogenate), and, after degassing, was centrifuged at 450 × *g* for 10 min to remove unbroken cells and large particulate material (e.g. intact brush borders). Less than 10% of the ($Na^+ + K^+$)-ATPase sedimented at this force, while up to 40% of the sucrase and 20% of the succinate dehydrogenase were recovered from the 450 × *g* pellet. The basolateral membranes were recovered from the 450 × *g* supernatant by centrifugation at 100 000 × *g* for 30 min: 50—60% of the protein, and less than 5% of the ($Na^+ + K^+$)-ATPase, sucrase and succinate dehydrogenase remained in the 100 000 × *g* supernatant. The pellet (P_2) was resuspended in 12.5% sorbitol buffered with 5 mM histidine/imidazole at pH 7.5 using 20 strokes of a motor driven glass-Teflon homogeniser. The suspension was layered on a 25—65% linear sorbitol gradient and centrifuged overnight using a Beckman SW 27 rotor [9]. The gradient was fractionated in 2-ml aliquots using an Auto-Densi-Flow apparatus (Buchler, Fort Lee, NJ). Each fraction was assayed for ($Na^+ + K^+$)-ATPase, sucrase and succinate dehydrogenase at 22° C as described [9] and protein was assayed by a commercial reagent kit (Bio-Rad, Richmond, CA). In five experiments on the mid-jejunum the initial specific activities were 1.1 ± 0.2, 0.68 ± 0.17 and 0.11 ± 0.02 $\mu mol \cdot mg^{-1} \cdot h^{-1}$ for sucrase, ($Na^+ + K^+$)-ATPase and succinate dehydrogenase, respectively, and the recoveries for protein, sucrase, ($Na^+ + K^+$)-ATPase and succinate dehydrogenase were 94 ± 4, 87 ± 2, 87 ± 4 and 84 ± 4%, respectively.

Basolateral membranes, identified by the ($Na^+ + K^+$)-ATPase activity [9], were harvested from the gradient fractions by dilution with 4 vols. of 5% sorbitol and centrifuged at 100 000 × *g* for 30 min. The membranes were then resuspended in 250 mM sorbitol to give protein concentrations of 4.5—6 mg/ml.

Transport of sugars into the basolateral membrane vesicles was measured using radioactive tracers and a rapid filtration technique. 20 µl of the membrane suspension was added to 160 µl of buffer at zero time and uptake into the vesicles was terminated by the addition of 50 µl of the reaction mixture to 1 ml of cold (0° C) stopping solution. The solution was then filtered through a prewashed, prechilled 0.45 µm pore size filter (Schleicher and Schuell, Keene, NH) and washed with 4.5 ml of the cold stopping buffer. The filters were then dissolved and assayed for radioactivity using a liquid scintillation counter. All experiments were carried out with dual-label techniques (^3H and ^{14}C) and all counts were corrected for quench, spillover and background. Radioactive isotopes, ^3H-labelled L-glucose, D-mannitol and D-glucose and ^{14}C-labelled L-glucose, D-glucose, D-mannitol and 3-*O*-methyl-D-glucose, were obtained from New England Nuclear, Boston, MA, Amersham, Arlington Heights, IL and ICN Pharmaceuticals, Irvine, CA. Aliquots of the isotopes, as supplied by the manufacturer, were dried down with a stream of dry nitrogen gas and then dissolved in 250 mM sorbitol. Appropriate mixtures of the isotopes were then added to the incubation media.

Sorbitol density gradient solutions were all buffered with 5 mM histidine/imidazole at pH 7.5 and contained 0.5 mM sodium EDTA. Membranes were suspended in a 250 mM sorbitol solution containing 5 mM NaN_3 and buffered

with 10 mM Hepes at pH 7.4. Most uptake experiments were carried out in the same media, but in some experiments mentioned specifically in the text sorbitol was partially replaced by sugars or salts. In a few experiments NaN_3 was omitted and the Hepes buffer was replaced by 5 mM Tris/Hepes, pH 7.4. The stopping solution contained 1 mM $HgCl_2$ in addition to 250 mM sorbitol, 5 mM NaN_3 and 5 mM Tris/Hepes (pH 7.4). Sugars were obtained in their purest form from J.T. Baker, Calbiochem, Sigma, Eastman Kodak, and Koch-Light Chemical companies.

All steps of the membrane isolation were carried out at 0—4° C, and transport experiments were performed at temperatures ranging from 0 to 37° C.

Results

Isolation of basolateral membranes

The distribution of basolateral membranes, brush border membranes, mitochondria and protein on the linear sorbitol density gradients in one experiment are shown in Fig. 1. Earlier work established that $(Na^+ + K^+)$-ATPase and sucrase were good markers for the basolateral and brush border membranes, respectively [9]. $(Na^+ + K^+)$-ATPase activity peaked near the low-density region of the gradient (fraction No. 4) while the sucrase came to equilibrium at the high-density end (fraction No. 10) of the gradient. The mitochondria have very similar densities to the brush border membranes and so the peak of succinate dehydrogenase activity was in fraction 9. 51% of the initial $(Na^+ + K^+)$-ATPase activity was present in the first seven fractions of the gradient and the specific activities of the peak fractions in this experiment were ten times greater than the specific activity of this enzyme in the initial homogenate. On the other hand the specific activities of both the sucrase and the succinate dehydrogenase are reduced to one third of the initial specific activities in the homogenate, i.e. the basolateral membranes were purified 30-fold with respect to the brush borders and mitochondria in the initial homogenate. The total recoveries of the enzymes and protein in this experiment ranged between 75 and 88%, i.e. there was little evidence of enzyme activation or inhibition during the the basolateral isolation procedure. These results are very similar to those obtained in our laboratory with other preparations of intestinal epithelial cells [9—13].

Sugar uptake into basolateral membrane vesicles

The uptake of D- and L-glucose into basolateral membranes recovered from fractions 2—7 was assayed using L-[^{3}H]glucose and D-[^{14}C]glucose and a filtration technique. Preliminary experiments revealed that the stereospecific uptake of glucose was maximal in fractions 2—4 and so in all subsequent work these fractions were pooled for transport experiments. One experiment showing the uptake of D- and L-glucose (2.3 mM) is presented in Fig. 2. The uptake, in nmol · mg^{-1} (protein) is plotted against time of incubation. D-Glucose equilibrated within the vesicles faster than the optical isomer; the half-time for D-glucose was 0.1 min compared with 2.4 min for L-glucose, i.e. the equilibration of the natural isomer with the intravesicular space was about 25 times faster than L-glucose. In this experiment the initial rate of D-glucose uptake

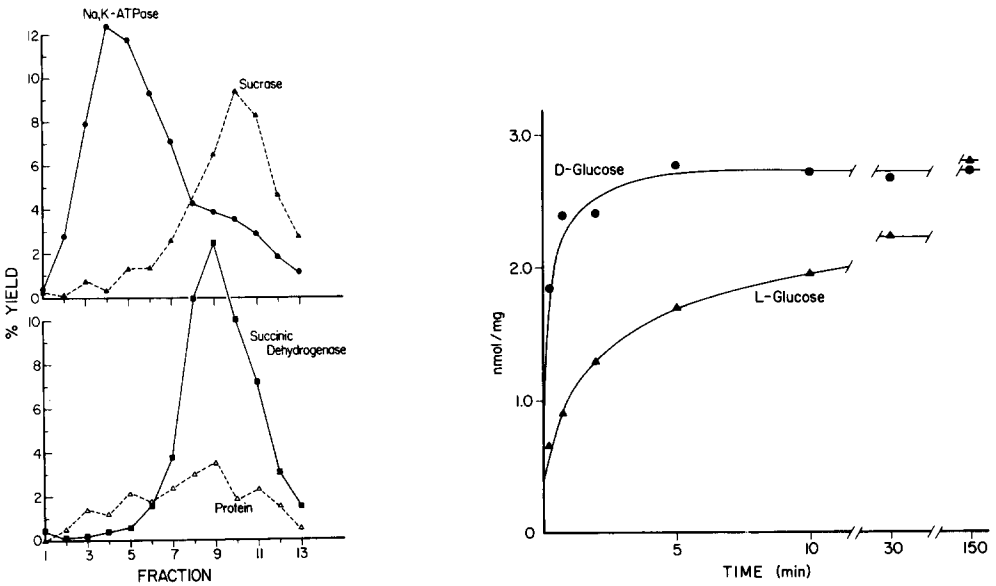

Fig. 1. The distribution of basolateral membranes ($(Na^+ + K^+)$-ATPase), brush border membranes (sucrase), mitochondria (succinate dehydrogenase) and protein on a linear density gradient. The pellet P_2 was resuspended in 12.5% sorbitol and layered on top of a 25—65% continuous sorbitol gradient. The sample was covered with buffer and then spun 12 h at 90 000 × *g* using a SW27 Beckman rotor. Fraction 1 contained the original sample and the overlay, and fractions 2—13 (2 ml each) corresponded to the linear 25—65% sorbitol gradient. The amount in each fraction is plotted as the percentage of the total amount of the enzyme or protein in the initial homogenate, i.e. percent yield.

Fig. 2. The uptake of D-[^{14}C]glucose and L-[^{3}H]glucose, in nmol · mg^{-1}, at 22° C plotted against time. Note that the amounts of the two isomers in the vesicles at the steady state (after 2,5 h incubation) were identical and that D-glucose equilibrated within the vesicle faster than L-glucose. The concentration of both sugars was 2.3 mM and the buffer composition inside and outside the vesicles was identical, i.e. 250 mM sorbitol, 5 mM NaN_3 buffered at pH 7.4 with 5 mM Hepes. Identical results were obtained when either the radioactive isotopes on the sugars was reversed or the buffers were free of sodium (250 mM sorbitol buffered with 5 mM Tris/Hepes to pH 7.4).

(measured at 0.25 min) was three times faster than that for L-glucose. In nine different experiments the initial rate of stereospecific D-glucose uptake was 7.1 ± 1.1 nmol · mg^{-1} · min^{-1}, and the steady-state amounts of D- and L-glucose within the vesicles amounted to 5.1 ± 0.7 and 4.9 ± 0.7 nmol · mg^{-1}, respectively. Identical equilibrium spaces were found for D-glucose, L-glucose, mannitol, 3-*O*-methyl-D-glucose and D-alanine. Although the equilibrium spaces were similar to that obtained by Hopfer and coworkers for intestinal basolateral membranes [7,8], the initial stereospecific uptake of glucose was about 3.5 times greater in the present experiments.

The rate of glucose transport was found to be constant within the range of 15—60 μg protein per filter, and there was no significant decline in the transport capacity of the membranes over the first 30 h when they were stored in sorbitol at 0° C.

Effect of temperature

The initial uptake of D-glucose and L-glucose into the basolateral vesicles (at 0.25 min) was measured over the temperature range 0—37° C. Arrhenius plots [log rate/(1/T)] of the results are presented in Fig. 3. The rate of L-glucose transport increased with temperature and with an activation energy of 3.1 kcal · mol^{-1}. A more complex relationship was observed with D-glucose; over the range 0—15°C an activation energy of 7.6 kcal · mol^{-1} was obtained, but above 15° C the activation energy approached the value for L-glucose. Fig. 3 shows the Arrhenius plot for the carrier-mediated uptake of D-glucose, i.e. the rate of D-glucose uptake minus the rate of L-glucose uptake, and over the low-temperature range the slope corresponded to an activation energy of 13.7 kcal · mol^{-1}.

Kinetics of D-glucose uptake

The initial rate of D- and L-glucose uptake (at 0.25 min) was measured as a function of concentration from 0.1 to 150 mM at 10° C to obtain an estimate of the transport kinetics (Fig. 4). The osmolarity of the incubation medium was kept constant throughout by varying the sorbitol concentration. The rate of L-glucose uptake was linear over the entire concentration range but the uptake of D-glucose showed both saturable and linear components. The passive permeabilities of the membrane vesicles to D-glucose, L-glucose and D-mannitol (not shown) were identical, i.e. the slopes of the linear components were superimposable. The stereospecific transport of D-glucose exhibited a V of approx. 120 nmol · min^{-1} · mg^{-1} and a K_m of approx. 44 mM. In the three experiments at 10° C the K_m ranged from 25 to 50 mM.

In one experiment the K_m and V for 3-*O*-methyl-D-glucose transport at 10° C were approx. 40 mM and 100 nmol · min^{-1} · mg^{-1}.

Specificity of sugar transport

This was studied by measuring the effect of 100 mM sugars on the initial rate of 2.3 mM D-glucose uptake into the membrane vesicles at 22° C (Table I). Among the 18 pyranose sugars tested, only L-glucose and α-methyl-D-glucoside failed to produce any significant inhibition. All other 16 sugars are either pentoses or hexoses with the D-glucose chair conformation (C1). α-Methyl-D-glucoside also has the C1 conformation and the lack of effect is due either to: (i) an hydroxyl group is required on carbon No. 1 or, (ii) the OCH_3 group is too bulky to allow the sugar to interact with the sugar carrier. It is of particular note that the OH group in the equatorial plane of the ring at carbon No. 2 is not an essential feature of the sugars that inhibit D-glucose transport: D-mannose, 2-deoxy-D-glucose, 2-deoxy-D-galactose, and 2-deoxy-D-allose all produce inhibition. An hydroxyl group in this position is essential for sodium cotransport of sugars in brush border membranes.

The fact that 3-*O*-methyl-D-glucose and 3-*O*-benzyl-D-glucose inhibit D-glucose transport indicates that an equatorial OH group on carbon No. 3 is not essential, and likewise the effects of D-galactose and L-arabinose indicates that the equatorial OH group on carbon No. 4 is not essential.

Furthermore, the nature of the substituent group on carbon No. 5, CH_3,

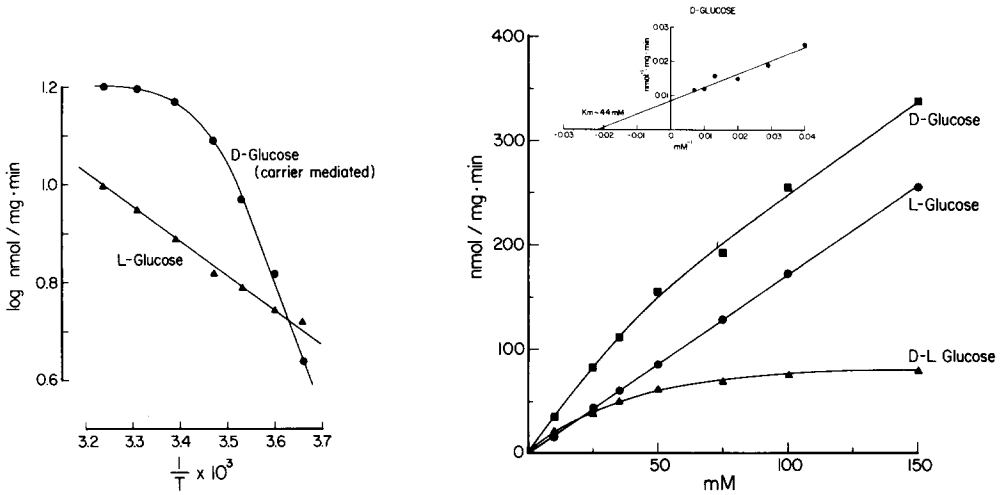

Fig. 3. The effect of temperature on the initial rate of L-glucose and D-glucose uptake into basolateral membrane vesicles. The results are presented in the form of an Arrhenius plot, i.e. log rate (nmol · mg^{-1} · min^{-1}) against $1/T$. Membranes were incubated at each temperature for 0.25 min in the presence of 2.3 mM D- and L-glucose. All uptakes were obtained in duplicate, and the data shown in this figure was obtained from one experiment. The rate of D-glucose uptake is shown as the rate of the stereospecific process, i.e. D-glucose — L-glucose. The data points for L-glucose were fitted to a straight line with a slope corresponding to an activation energy of 3.1 kcal · mol^{-1}. Below 15°C the slope of the curve for D-glucose uptake gave an activation energy of 13.7 kcal · mol^{-1}, but above 15°C curve became non-linear and the activation energy approached that for L-glucose permeation.

Fig. 4. The kinetics of D- and L-glucose uptake into basolateral membrane vesicles. The initial rate of uptake (nmol · mg^{-1} · min^{-1}) was measured at 10°C by incubating the vesicles for 0.25 min. The osmolarity of the incubation media was kept constant by substituting D- or L-glucose for sorbitol on a mol for mol basis. Fluxes were estimated in duplicate at each sugar concentration in 2—6 experiments. The S.E. for each mean was less than 20%. The rate of L-glucose uptake was linear from 10 to 150 mM in this experiment, but the uptake of D-glucose exhibited two components: a saturable and a linear component. The linear component, seen above 75 mM, was parallel to the L-glucose and D-mannitol (not shown) slopes. The stereospecific uptake of glucose (D-glucose — L-glucose) saturated at 75—100 mM, and a plot of 1/rate vs. 1/concentration (insert) yield a K_m and V of approx. 44 mM and approx. 120 nmol · mg^{-1} · min^{-1}, respectively.

CH_2OH and CH_2OCH_3, does not seriously effect the ability of pyranose sugars to interact with the D-glucose transport system. Finally, the observation that 2,6-dideoxy-D-allose (digitoxose, 2,6-dideoxy-D-ribose hexose) inhibits D-glucose uptake 29% suggests that a pyranose ring with a minimum of two hydroxyl groups in the equatorial plane of the ring (carbon Nos. 1 and 4) is able to interact with the sugar carrier in the intestinal basolateral membranes.

A similar experiment at 22° C also demonstrated that the specific uptake of 3-*O*-methyl-D-glucose could also be inhibited by D-glucose and 3-*O*-methyl-D-glucose: the uptake of 0.5 mM 3-*O*-methyl-D-glucose was inhibited 19 and 53%, respectively.

Effect of inhibitors

The uptake of D- and L-glucose into the membrane vesicles in the presence and absence of 1 mM $HgCl_2$ is shown in Fig. 5. Hg^{2+} eliminated the stereo-

TABLE I

SPECIFICITY OF SUGAR TRANSPORT

All experiments were carried out in duplicate at 22°C. The stereospecific transport of glucose was obtained from the uptake of 2.3 mM D- and L-glucose at 0.25 min. All sugars were tested for inhibition at 100 mM and the control was 100 mM D-mannitol. In the case of multiple experiments the mean value ± S.E. and the number of runs (in parenthesis) are given.

Sugar	% inhibition
D-Glucose	42 ± 4 (8)
L-Glucose	0
α-Methyl-D-glucose	0
2-Deoxy-D-glucose	42 ± 3 (3)
D-Mannose	25 ± 4 (4)
N-Acetyl-D-glucosamine	16
3-*O*-Methyl-D-glucose	67 ± 3 (7)
3-*O*-Benzyl-D-glucose	61 ± 2 (3)
D-Galactose	22 ± 7 (7)
2-Deoxy-D-galactose	22
6-Deoxy-D-glucose	37 ± 2 (3)
6-Deoxy-D-galactose	53
6-*O*-Methyl-D-galactose	10 ± 7 (3)
D-Xylose	30
L-Arabinose	21
D-Ribose	49
2-Deoxy-D-allose	37
2,6-Dideoxy-allose	29

specific uptake of D-glucose; there was also a small decrease in the initial rate of L-glucose uptake and the amount of both isomers in the vesicles at equilibrium was 20% higher than in controls. In agreement with Hopfer et al. [8] we observed that phlorrhizin, cytochalasin B and phloretin inhibit glucose uptake: 0.5 mM phloretin, 10 μM cytochalasin B and 0.1 mM phlorrhizin inhibited the stereospecific uptake 100%, 51% and 17%, respectively, in one experiment.

Glucose transport and sodium gradients

The effect of a sodium concentration gradient on the basolateral glucose transport system was tested by substituting 100 mM NaCl or KCl for 200 mM sorbitol in the uptake media (Fig. 6). There was no specific effect of the sodium gradient on the rate of D- and L-glucose transport into these basolateral membrane vesicles. Identical results were obtained when the vesicles were first preloaded with 100 mM KCl and then incubated in solutions containing D-[^{14}C]glucose and L-[^{3}H]glucose and either 100 mM NaCl or 100 mM KCl. These observations are consistent with those of Hopfer et al. [8]. The earlier reported stimulation of glucose transport by sodium gradients in basolateral membranes [7] is accounted for by contamination of the preparation by brush border membranes (the basolateral membranes were only enriched 5-fold and there was no clear statement about the contamination by brush borders).

Comparison of basolateral membranes from jejunum and ileum

In two experiments we prepared basolateral membranes from the duo-

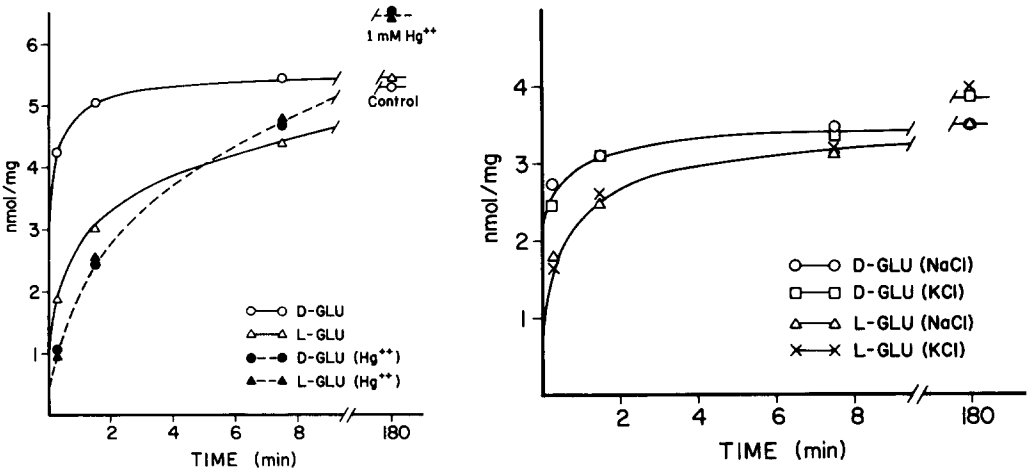

Fig. 5. The effect of 1 mM $HgCl_2$ on the uptake of D- and L-glucose into basolateral membrane vesicles. The stereospecific uptake of glucose into the vesicles was blocked by the Hg^{2+}. Note that there was a slight inhibition of L-glucose uptake, but that there was a 20% increase in the sugar 'space' within the vesicles in the presence of the inhibitor.

Fig. 6. The effect of a NaCl gradient on the uptake of D- and L-glucose into basolateral membrane vesicles. In these experiments the vesicles contained the 250 mM sorbitol buffer and at zero time they were added to an incubation mix containing 100 mM NaCl, 50 mM sorbitol, 2.3 mM D- and L-glucose buffered at pH 7.4 with 5 mM Hepes. In the control experiment uptake was measured in the presence of a 100 mM KCl gradient.

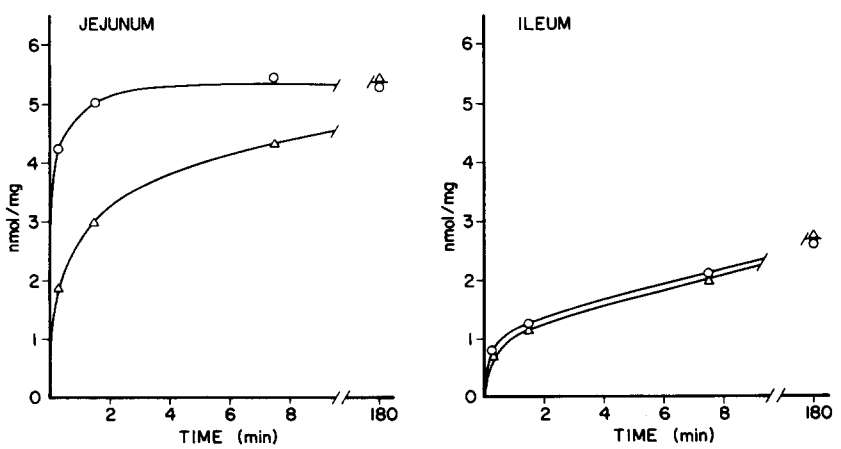

Fig. 7. Comparison of stereospecific glucose uptake by basolateral membrane prepared from the jejunum and ileum. In this experiment basolateral membranes were obtained from mature enterocytes in the proximal duodenum, mid-jejunum, and terminal ileum from the same animal. The membranes were incubated in the sorbitol buffer containing 2.3 mM D- and L-glucose and uptake was measured as a function of time. Similar results were obtained for the membranes prepared from the duodenum and jejunum (not shown) in that the rate of D-glucose uptake was about 25 times faster than that for L-glucose. In the ileum there was no significant difference between the rates of D- and L-glucose uptakes Note that the glucose space in the ileum was less than half of that in the jejunum.

denum, mid-jejunum, and the terminal ileum. The uptake of D-glucose and L-glucose into membranes from the jejunum and ileum in one of these experiments are shown in Fig. 7. In the ileum there was no stereospecific uptake of glucose which is in marked contrast to that observed for the membranes prepared from the duodenum and jejunum.

Discussion

In this laboratory we have developed procedures for the isolation of intestinal basolateral membranes [9—13]. These give high yields (30—65%) of basolateral membranes that are enriched 15-fold and are free of contamination by brush border membranes, mitochondria, nuclei and lysosomes. This paper reports on the use of these isolated plasma membrane vesicles to study sugar transport across the basolateral surface of the epithelium. We have confirmed and extended the preliminary observations of Hopfer and coworkers [7,8] and we conclude that sugar uptake by basolateral membrane vesicles occurs by a facilitated diffusion mechanism that is reminiscent of that described for the human red blood cell.

Facilitated sugar transport exhibits the following characteristics:

1. Transport is stereospecific. D-Glucose equilibrates within the basolateral vesicles with a half-time 1/25th that for L-glucose, and the initial rate of D-glucose uptake is three times faster than for its optical isomer (Fig. 3).

2. The driving force for transport is the sugar concentration gradient. Even though the rate of D-glucose uptake is greater than either L-glucose or mannitol, the amount of each solute in the vesicle at the steady state was identical (Fig. 3). Furthermore, the rate of D-glucose transport was unaffected by sodium gradients (Fig. 6).

3. Transport shows saturation kinetics. The K_m and V for stereospecific D-glucose transport were approx. 44 mM and 110 nmol $\cdot$ mg^{-1} $\cdot$ min^{-1}, respectively (Fig. 4).

4. Competition occurs between structural analogs. D-Glucose uptake was inhibited by 15 other sugars (Table I).

5. Inhibitors block transport. 0.5 mM phloretin and 1 mM Hg^{2+} eliminate the stereospecific uptake of glucose (Fig. 5) while cytochalasin B and phlorrhizin produce significant inhibitions.

This transport process is different from the sodium cotransport process that occurs in the brush border membranes. First, facilitated diffusion is less specific, i.e. all sugars with the pyranose ring conformation of D-glucose are transported, but in the case of sodium cotransport additional specificity is conferred by the requirement that only hexoses with an equatorial -OH group on carbon No. 2 of the ring can use the carrier. Second, the rate of sodium cotransport depends on the magnitude of the sodium electrochemical potential gradient [2], whereas for the facilitated diffusion process in the basolateral membranes the rate is independent of the presence or absence of sodium gradients. Finally, sodium cotransport is much more sensitive to phlorrhizin than its aglycone phloretin.

The specificity of the sugar transport system in basolateral membranes is worthy of comment. All the sugars that compete with D-glucose are either pen-

toses or hexoses with a pyranose ring in the same chair conformation as D-glucose, i.e. the C1 chair conformation (see Refs. 16 and 17). This suggests that the sugar carrier is able to distinguish between the three-dimensional conformations of the two chair conformations of the pyranose ring. In red cell and choroid plexus the binding of the sugar to the carrier is promoted by the presence of hydroxyl groups in the equatorial plane of the pyranose ring; the more equatorial groups the lower the K_m for sugar transport. This is also probably true for the basolateral membranes. Inspection of the data on competition (Table I) indicates that, with the possible exception of the hydroxyl group at carbon No. 1, there is no unique requirement for an equatorial -OH group at any given position in the pyranose ring. This is unlike the sodium cotransport system that required an equatorial -OH group at carbon No. 2. In the case of α-methyl-D-glucoside either the free -OH group may be necessary for binding with the carrier or, the $-OCH_3$ group may cause steric hindrance. There is no evidence of steric hindrance with $NHCOCH_3$ at carbon No. 2, with $-OCH_3$ or $OCH_2C_6H_5$ at carbon No. 3 or with $-CH_2OCH_3$ at carbon No. 5. It should be noted that Bihler and Cybulsky [3] also found that α-methyl-D-glucoside did not compete for 3-*O*-methyl-D-glucose uptake in isolated cells. Sugars with only two equatorial -OH groups appear to be capable of interacting with the carrier in basolateral vesicles as 2,6-dideoxy-D-allose inhibits glucose uptake.

Although the K_m observed for glucose uptake by the basolateral membrane vesicles appears to be high (approx. 44 mM), it should be noted that these experiments were carried out at 10° C in order to obtain a more accurate measure of the initial rate of sugar transport. The K_m for facilitated glucose transport in red blood cells has an activation energy of 10 kcal · mol^{-1} [18], i.e. comparable to the activation energies obtained here for the initial rate of glucose uptake at a concentration far below the K_m. This suggests that at 37° C the K_m for D-glucose transport across intestinal basolateral membranes is approx. 10 mM. This could not be tested owing to the difficulty of obtaining initial rates of transport at high temperatures with the present technique.

The results obtained in this study are essentially in agreement with the preliminary data obtained by Hopfer and coworkers [7,8] for transport of D-glucose into rat intestinal basolateral membranes. The reported 22% stimulation of D-glucose transport sodium gradients [7] is most likely due to contamination of their basolateral membrane vesicles with brush borders. The sugar transport mechanism also appears to be similar in both the intestinal and renal basolateral membrane vesicles. Preliminary studies of rat renal basolateral membranes [19] showed that: (i) D-glucose uptake was faster than L-glucose; (ii) phloretin was a more potent inhibitor than phlorrhizin, and (iii) the activation energy D-glucose transport was more than 8 kcal · mol^{-1} below 16° C, and above this temperature the activation energy approached 3 kcal · mol^{-1}. (The origin for the 'phase transition' in both renal and intestinal membranes is not quite clear at this time. However, the break in the Arrhenius plots may be related to the fact that the method used to measure the initial rate of uptake is questionable at the higher temperatures, e.g. in our experiments the intravesicular space was already 60—70% full with D-glucose after 15 s when the temperature of the incubation media was 22° C.)

The sugar transport system in renal basolateral membranes showed a sodium

sensitivity reminiscent of that reported previously for intestinal basolateral membranes [7]. The explanation is probably the same, i.e. contamination of their basolateral membranes with brush borders.

Detailed studies on the transport properties of basolateral membranes in the intact epithelium have been hampered by the inaccessibility of the serosal surface of the epithelium owing to the presence of rather thick layers of muscle and connective tissue. By way of contrast much more is known about the transport mechanisms of the brush border membrane in the intact epithelium as a result of unidirectional influx measurements (see Ref. 1). It is well established that the major pathway for sugar transport in the brush border is via the sodium cotransport system, and this has been confirmed by uptake studies on isolated brush border membranes vesicles (see Refs. 2,7 and 8).

An ingenious approach to the problem of sugar transport across the basolateral surface of the intact epithelium was devised by Bihler and Cybulsky [3]. They treated the brush border surface of the intact small intestine with an irreversible inhibitor ($HgCl_2$) and then studied uptake of sugars into isolated cells. It was established that sugar uptake by the isolated cells was insensitive to sodium and 0.5 mM phlorrhizin, and that the specificity of the processes differed from that at the brush border membrane. Apart from the phlorrhizin experiment, their observations are consistent with our results. Their lack of effect of phlorrhizin is probably due to the fact that they did not measure initial rates of sugar uptake into cells; they measured uptake after 10 min incubations and after this time, even with marked phlorrhizin inhibition, the sugars should have equilibrated with the intracellular compartment. It is assumed that sugar uptake into the Hg^{2+}-treated cells occurred across the basolateral aspect of the cell. However, it should be noted that the Hg^{2+} treatment did not completely eliminate sugar uptake across the brush border surface.

Kimmich and his collaborators have also used isolated cells from the chick intestine to study mechanisms of sugar transport [4—6]. They have used two approaches: the first was to measure sugar uptake in the absence of sodium, the rationale being that under these conditions the sodium cotransport system in the brush border is inoperative, and the second was to study the uptake of 2-deoxy-D-glucose, a sugar which is unable to utilize the sodium cotransport system in the brush border. They found that sugar transport into the cells under these conditions was: (i) saturable. The K_m values for 3-*O*-methyl-D-glucose and 2-deoxy-D-glucose transport were greater than 70 mM and 27 mM, respectively, and the V for both sugars was about 40 nmol $\cdot$ mg^{-1} $\cdot$ min^{-1} at 37° C. The K_m values were of the same order of magnitude as we observed for the rat basolateral membrane vesicles at 10° C; (ii) uptake was more sensitive to phloretin than phlorrhizin; (iii) uptake was also inhibited by cytochalasin B and theophylline; (iv) 2-deoxy-D-glucose uptake was insensitive to the presence of sodium, and (v) there was broad substrate specificity for transport, e.g. 2-deoxy-D-glucose uptake was inhibited by other sugars in the sequence glucose > mannose > galactose > 3-*O*-methyl-D-glucose > xylose > fructose >> fucose, ribose. These characteristics are very similar to those described above for basolateral membrane vesicles.

Although Kimmich's group have no direct evidence for the route of sugar uptake, they favor uptake via the basolateral membrane. Undoubtedly some,

but not necessarily all, facilitated sugar uptake into isolated intestinal cells occurs through the basolateral membrane. In renal brush border membrane vesicles the sodium-independent sugar transport process has been well studied [20,21], and the rate of facilitated diffusion ranges between 10 and 20% of the total uptake.

In conclusion, direct studies of sugar transport by intestinal basolateral membrane vesicles has clearly established that the major transport mechanism is facilitated diffusion. This confirms the tentative conclusions drawn earlier on the basis of studies with isolated cells. The significance of this basolateral membrane sugar transport system is that it provides for the second step, the exit step, in the overall absorption of sugars from the gut lumen, and, in addition, when the glucose content of the diet is low the metabolic demands of the epithelium are met by the entry of glucose from the blood across the basolateral membrane.

Acknowledgements

These studies were supported by USPHS grants AM 19567 and NS 09666.

References

1 Schultz, S.G. and Curran, P.F. (1970) Physiol. Rev. 50, 637—718
2 Murer, H. and Hopfer, U. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 484—488
3 Bihler, I. and Cybulsky, R. (1973) Biochim. Biophys. Acta 298, 429—437
4 Kimmich, G.A. and Randles, J. (1975) J. Membrane Biol. 23, 57—76
5 Kimmich, G.A. and Randles, J. (1976) J. Membrane Biol. 27, 363—379
6 Randles, J. and Kimmich, G.A. (1978) Am. J. Physiol. 234, C64—C72
7 Murer, H., Hopfer, U., Kinne-Saffran, E. and Kinne, R. (1974) Biochim. Biophys. Acta 345, 170—179
8 Hopfer, U., Sigrist-Nelson, K., Ammann, E. and Murer, H. (1976) J. Cell. Physiol 89, 805—810
9 Mircheff, A.K. and Wright, E.M. (1976) J. Membrane Biol. 28, 309—333
10 Van Os, C.H., Mircheff, A.K. and Wright, E.M. (1977) J. Cell Biol. 73, 257—260
11 Mircheff, A.K., van Os, C.H. and Wright, E.M. (1978) Membrane Biochem. 1, 177—185
12 Mircheff, A.K., Hanna, S.D., Walling, M.W. and Wright, E.M. (1979) Prep. Biochem. 9, 133—148
13 Walling, M.W., Mircheff, A.K., van Os, C.H. and Wright, E.M. (1978) Am. J. Physiol. 235, E539—545
14 Stern, B.K. (1966) Gastroenterology 51, 855—867
15 Douglas, A.P., Kerley, R. and Isselbacher, K.J. (1972) Biochem. J. 128, 1329—1338
16 LeFreve, P.G. (1961) Pharmacol. Rev. 13, 39—70
17 Prather, J.W. and Wright, E.M. (1970) J. Membrane Biol. 2, 150—172
18 Sen, A.K. and Widdas, W.F. (1962) J. Physiol. 160, 392—403
19 Kinne, R., Murer, H., Kinne-Saffran, E., Thees, M. and Sachs, G. (1975) J. Membrane Biol. 21, 375—395
20 Turner, R.J. and Silverman, M. (1978) Biochim. Biophys. Acta 507, 305—321
21 Turner, R.J. and Silverman, M. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 2825—2829