Biochem. J. (1986) 237, 229–234 (Printed in Great Britain)

Aboral changes in D-glucose transport by human intestinal brush-border membrane vesicles

Michael K. BLUETT, Naji N. ABUMRAD, Noushin ARAB and Fayez K. GHISHAN*
Vanderbilt University Medical Center, Departments of Surgery and Pediatrics, Nashville, TN 37232, U.S.A.

D-Glucose transport was investigated in isolated brush-border membrane vesicles from human small intestine. Characteristics of D-glucose transport from the jejunum were compared with that in the mid and terminal ileum. Jejunal and mid-ileal D-glucose transport was Na^+-dependent and electrogenic. The transient overshoot of jejunal D-glucose transport was significantly greater than corresponding values in mid-ileum. The terminal ileum did not exhibit Na^+-dependent D-glucose transport, but did exhibit Na^+-dependent taurocholate transport. Na^+–glucose co-transport activity as measured by tracer-exchange experiments was greatest in the jejunum, and diminished aborally. We conclude that D-glucose transport in man is Na^+-dependent and electrogenic in the proximal intestine and directly related to the activity of D-glucose–Na^+ transporters present in the brush-border membranes. D-Glucose transport in the terminal ileum resembles colonic transport of D-glucose.

INTRODUCTION

The absorption of D-glucose by the small intestine is a complex process representing the sequential transfer of glucose molecules across the luminal and contraluminal membranes of columnar epithelial cells. Crane (1962, 1965) first postulated that the energy required for glucose transport was derived from the flow of Na^+ down a gradient of chemical potential present across the brush-border membrane. These studies using whole tissue preparations have defined the overall picture of transport. More recently, isolated plasma-membrane vesicles have been used to study small-intestinal transport processes (Hopfer *et al.*, 1973). By isolating the luminal and the contraluminal plasma membranes, it was possible to detect a polar distribution of transport systems in the cell envelope. The major advantage of this approach is the possibility of pre-setting the experimental conditions at both sides of the membrane at will. Moreover, tissue metabolism of substrates is not involved. Hopfer and co-workers, using isolated brush-border membrane vesicles (BBMV) from rat small intestine, unequivocally demonstrated the coupling of Na^+ and D-glucose fluxes (Hopfer *et al.*, 1973; Murer & Hopfer, 1974). Moreover, phlorizin has been shown to inhibit the Na^+-dependent uptake of D-glucose (Hopfer *et al.*, 1973; Murer & Hopfer, 1974).

Numerous studies have shown that D-glucose is absorbed by all regions of the small intestine, but the jejunum is the region responsible for most D-glucose absorption. Hopfer *et al.* (1976), using rat intestinal BBMV, has shown jejunal transport of D-glucose to be 2–3 times faster than transport in the ileum. Some perfusion studies have shown that the ileum is unable to transport glucose against a concentration gradient (Love, 1968).

Most of the definitive studies which have characterized D-glucose transport have been performed in animals (Hopfer *et al.*, 1973, 1975; Murer *et al.*, 1974). A number of incubation techniques and perfusion studies have defined the overall process of D-glucose transport in man (Modiglinani & Bernier, 1971; Cook, 1973; Phillips & McGill, 1973). Studies *in vitro* using intact epithelial cells from human mucosal biopsies have been successful in diagnosing specific defects responsible for specific malabsorption states (Wimberly *et al.*, 1973). Only one study has examined D-glucose transport in man by using BBMV (Lucke *et al.*, 1978).

More recently, investigators have focused on the longitudinal specialization of human small intestine and have shown that the intestinal brush-border membrane disaccharidases, sucrase and lactase, have the greatest activity in the proximal jejunum (Triadon *et al.*, 1983). However, the aboral changes in D-glucose transport at the membrane level are unknown in man. Thus we investigated D-glucose transport across BBMV prepared from segments of normal jejunum and mid and terminal (near the ileocaecal valve) ileum of man.

MATERIALS AND METHODS

Materials

D-[6-^{3}H(n)]Glucose (33.1 Ci/mmol) was purchased from New England Nuclear Corp. (Boston, MA, U.S.A.). Enzymes and substrates for leucine aminopeptidase were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Cellulose nitrate filters (0.45 μm pore size) were obtained from Sartorius Filters (Hayward, CA, U.S.A.). All other chemicals were of the highest purity available.

Selection of patients

Segments (5 cm long) of histologically normal jejunum, mid and terminal ileum adjacent to diseased segments were obtained during surgery. Permission of the Human Research Committee was obtained and informed consent was secured from the subjects. Six jejunum (five tumour, one obstruction), three mid-ileum (tumours) and three

Abbreviation used: BBMV, brush-border membrane vesicles.

* To whom all correspondence and reprint requests should be sent.

terminal ileum (two tumours, one obstruction) segments were obtained. One entire intestine was obtained from a transplant donor. Ages of patients were 35 to 55 years (mean 43 ± 7).

Isolation of brush-border membrane vesicles

Specimens were immediately rinsed with ice-cold Ringer's lactate solution. The mucosa was scraped from each segment, and BBMV were prepared by using a modified bivalent-cation precipitation method originally described for kidney BBMV (Evers *et al.*, 1978). Preparations were maintained at approx. 4 °C at all times. Mucosal scrapings were placed in 60 ml of mannitol buffer (300 mM-mannitol/5 mM-EGTA/12 mM-Tris/HCl, pH 7.1) and 240 ml of cold distilled water and then homogenized with a Waring-blender-type homogenizer at maximum speed. The homogenate was treated with 3 ml of 1 M-$MgCl_2$ and centrifuged at 3000 *g* for 15 min. The supernatant was then centrifuged at 27000 *g* for 30 min. The resulting pellet was resuspended in 60 ml of 60 mM-mannitol/5 mM-EGTA/12 mM-Tris/HCl (pH 7.1) and homogenized in a Potter–Elvehjem apparatus for ten strokes at the highest speed. The homogenate was treated with 0.6 ml of 1 M-$MgCl_2$ and centrifuged at 3000 *g* for 15 min. The supernatant was spun at 27000 *g* for 30 min. The pellet was resuspended in 30 ml of 250 mM-mannitol/20 mM-Hepes/Tris (pH 7.4) and homogenized with the Potter–Elvehjem apparatus for ten strokes at the highest speed. The suspension was centrifuged at 48400 *g* for 30 min. With a 25-gauge needle and syringe, the pellet was resuspended in the desired volume of transport buffer as described in the Figure legends. All centrifugation steps were carried out in a Beckman J2-21 rotor. A sample of each final BBMV preparation was removed for protein determination by the method of Lowry *et al.* (1951). Bovine serum albumin was used as a standard.

Purity of the membrane vesicle preparation

The purity of the membranes were assessed by the measurement of leucine aminopeptidase, an enzyme marker for intestinal brush-border membrane, with the Sigma kit no. 251. The procedure is based on the principle that leucine aminopeptidase cleaves a substrate L-leucyl-β-naphthylamide to leucine + β-naphthylamine, which can be measured spectrophotometrically. $(Na^+ + K^+)$-dependent ATPase was measured as described by Wilson & Treanor (1981). Cytochrome oxidase and NADPH:cytochrome *c* reductase were assayed as described by Beaufay *et al.* (1974).

Transport studies

Uptake of radiolabelled D-glucose by BBMV was measured by the membrane-filtration technique previously described (Hopfer *et al.*, 1973). The exact compositions of the incubation media are given in the legends to the Figures. Typically, membrane vesicles were adjusted to 200 mM-mannitol/20 mM-Tris/Hepes, pH 7.4 at 0 °C. Transport was initiated by adding 20 μl of membrane suspension to 100 μl of incubation media kept in a 37 °C water bath. The concentration of D-glucose was constant at 0.1 mM. At the desired time interval (20 s, 1, 2 and 20 min), a 20 μl sample was removed from the incubation suspension and diluted in 1 ml of ice-cold stop solution (100 mM-mannitol/100 mM-NaCl/10 mM-K_3PO_4/0.2 mM-phlorizin). The stop solution containing vesicles was immediately pipetted on to the middle of pre-wetted micropore filters (cellulose nitrate) and kept under suction. The filter was washed with 5 ml of ice-cold stop solution and then dissolved in Bray's solution (New England Nuclear). The radioactivity remaining on the filters was counted by standard liquid-scintillation techniques. All experiments were repeated in duplicate or triplicate with freshly prepared membranes. Absolute D-glucose uptake was expressed as pmol/mg of protein. Data were analysed by using Student's *t* test for unpaired data.

D-Glucose uptake, expressed as pmol/mg of vesicle protein, was calculated as follows:

$$\text{D-glucose uptake (pmol/mg of protein)} = \frac{\text{Radioactivity in vesicles on the filter (c.p.m.)} - \text{background radioactivity (c.p.m.) (blanks)}}{\text{Specific radioactivity} \times \text{vesicle protein in the filter (mg)}}$$

where 'background' represents radioactivity remaining in the filter after pipetting 20 μl of incubation media containing D-glucose tracer without vesicles, and specific radioactivity represents c.p.m. of a standard divided by amount of D-glucose.

RESULTS

Purity of the membrane vesicles

Table 1 depicts the specific activity of leucine aminopeptidase in mucosal homogenates and BBMV prepared from jejunum, mid-ileum and terminal ileum. There was a 20–26-fold enrichment of brush-border leucine aminopeptidase compared with total mucosal homogenate. There was either minimal or no enrichment of $(Na^+ + K^+)$-ATPase, of cytochrome oxidase or cytochrome *c* reductase enzymes, indicating minimal contamination with basolateral, mitochondrial or endoplasmic-reticulum systems respectively. Using a similar cation precipitation technique, we have obtained similar enrichment of these marker enzymes in the rat (Ghishan & Wilson, 1985). Similar enrichment of brush-border enzyme markers was reported by other investigators (Murer & Kinne, 1980). Brush-border membranes prepared by this method are 90% right-side-out (Murer & Kinne, 1980).

Transport properties

Jejunum. Fig. 1 depicts D-glucose uptake by jejunal BBMV. In the presence of a Na^+ gradient across the vesicle, there was a transient initial 'overshoot' at 20 s, compared with equilibrium uptake at 20 min. At the peak of 'overshoot', the accumulation of D-glucose was approx. 3 times the equilibrium value. By 20 min, the accumulation of D-glucose decreased to equilibrium values and reflected efflux from the vesicles. The 'overshoot' represents the transient accumulation of D-glucose in the vesicle space, driven by Na^+ gradient. Once the Na^+ gradient across the vesicles disappears, efflux of D-glucose occurs and values at 20 min represent equilibrium or vesicle size.

Table 1. Specific activity of marker enzymes in brush-border membrane vesicles and mucosal homogenate

For details, see the text. Values are means ± S.E.M. Numbers in parentheses indicate enrichment factor. Abbreviation: ND, not done.

	Jejunum		Mid-ileum		Terminal ileum	
	BBMV	Mucosa	BBMV	Mucosa	BBMV	Mucosa
Leucine aminopeptidase (units/mg of protein)	6110 ± 236	230 ± 20 (26)	4830 ± 310	210 ± 12 (23)	3330 ± 167	161 ± 16 (20)
$(Na^+ + K^+)$-ATPase (μmol/min per mg of protein)	0.47 ± 0.03	1.2 ± 0.1	ND	ND	1.7 ± 0.1	1.2 ± 0.1
Cytochrome *c* oxidase (μmol/min per mg of protein)	0.1 ± 0.02	1.1 ± 0.05	ND	ND	0.2 ± 0.05	3.0 ± 0.1
Cytochrome *c* reductase (μmol/min per mg of protein)	0.1 ± 0.02	0.1 ± 0.02	ND	ND	0.5 ± 0.01	0.5 ± 0.1

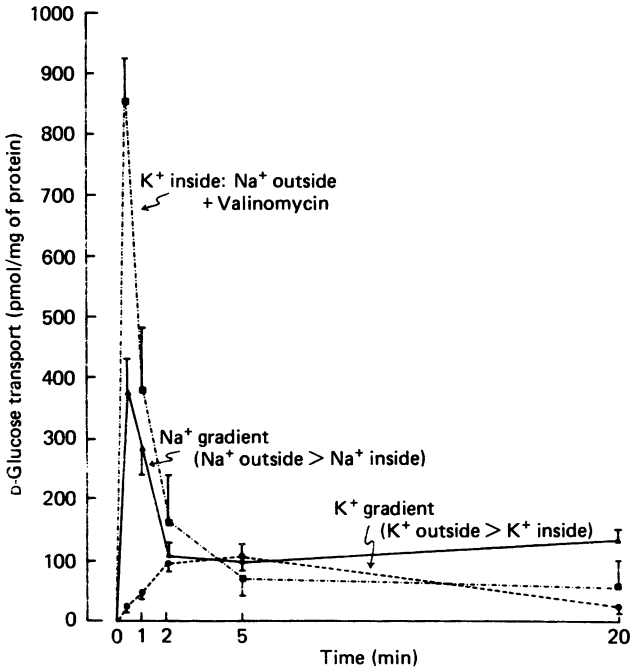

Fig. 1. D-Glucose uptake by brush-border membrane vesicles from human jejunum

Vesicles were prepared in 200 mM-mannitol/20 mM-Hepes/Tris buffer, pH 7.4. Transport was determined under three different conditions. ▲, Na^+ gradient (Na^+ outside > Na^+ inside). The reaction was started by the addition of 20 μl of vesicles to 100 μl of incubation medium containing 100 mM-mannitol, 100 mM-NaCl, 20 mM-Hepes/Tris buffer, pH 7.4, and 50 μl of D-[^{3}H]glucose. Temperature was maintained at 37 °C. ●, K^+ gradient (K^+ outside > K^+ inside). The reaction was started by the addition of 20 μl of vesicles to 100 μl of incubation medium containing 100 mM-KCl, 100 mM-mannitol, 20 mM-Hepes/Tris buffer, pH 7.4, and 50 μl of D-[^{3}H]glucose. Temperature was maintained at 37 °C. ■, Na^+ gradient (Na^+ outside > Na^+ inside) in the presence of valinomycin (10 μg/mg of protein). Vesicles were preincubated for 60 min at room temperature in 100 mM-KCl/100 mM-mannitol/20 mM-Hepes/Tris buffer, pH 7.4. The reaction was started by addition of 20 μl of vesicles to incubation medium containing 100 mM-NaCl, 100 mM-mannitol, 20 mM-Hepes/Tris, pH 7.4, valinomycin (100 μg/mg of protein) and 50 μl of D-[^{3}H]glucose. Temperature was maintained at 37 °C. Samples were taken at 0.3, 1, 2, 5 and 20 min. Values are means ± S.E.M. ($n = 6$).

The imposition of a K^+ gradient ([K^+] outside > [K^+] inside) resulted in no overshoot (Fig. 1). Equilibrium values were not significantly different ($P > 0.05$) from those in studies with a Na^+ gradient. Vesicular volume calculated from equilibrium values was 1.2 ± 0.2 μl/mg of protein. These values are similar to vesicular volumes obtained from rat jejunal BBMV (Murer & Kinne, 1980).

The role of the membrane electrical potential was examined by determining D-glucose uptake by BBMV under conditions in which membrane potentials were induced experimentally by the ionophore valinomycin. Valinomycin mediates the electrogenic movement of K^+ down its concentration gradient, thus setting up an electrical potential across the membrane (McLaughlin & Eisenberg, 1975; Wilson & Treanor, 1981). When BBMV were preincubated with K^+ and then assayed for Na^+-dependent glucose transport in the presence of valinomycin, a greatly enhanced 'overshoot' resulted (Fig. 1). The development of this electrical potential accelerated the influx of Na^+ and D-glucose, indicating that D-glucose uptake is driven by the imposed electrical potential.

Mid-ileum. Fig. 2 depicts D-glucose transport by mid-ileal BBMV. In the presence of a Na^+ gradient across the vesicle, the 'overshoot' phenomenon occurred. The peak 'overshoot' accumulation of D-glucose was approximately twice the equilibrium value. The 'overshoot' values in mid-ileum were significantly less than corresponding values in the jejunum ($P < 0.05$). The presence of the K^+ gradient resulted in no 'overshoot' uptake of D-glucose.

Terminal ileum. Fig. 3 depicts the D-glucose uptake by BBMV prepared from terminal ileum (near the ileocaecal valve). No 'overshoot' accumulation of D-glucose occurred in the presence of a Na^+ gradient, and initial uptake was not significantly different from equilibrium values ($P > 0.05$). The uptake of D-glucose at 20 s in the presence of a Na^+ gradient did not differ from the transport that occurred in the presence of the K^+ gradient ($P > 0.05$). Moreover, uptake experiments were done at 25 °C to determine whether this finding is related to the conditions set. Uptakes at 20 s, 1, 2, 5 and 20 min were 70 ± 5, 75 ± 2, 68 ± 2, 80 ± 6 and 100 ± 12 pmol/mg of protein respectively. These values are not different from uptake values at 37 °C (Fig. 3). Furthermore, to determine whether segments of the terminal ileum are

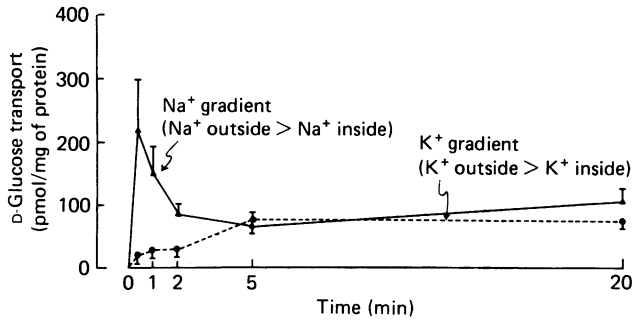

Fig. 2. D-Glucose uptake by brush-border membrane vesicles from human mid-ileum

Vesicles were prepared in 200 mM-mannitol/20 mM-Hepes/Tris buffer, pH 7.4. Transport was determined under two different conditions. ▲, Na^+ gradient (Na^+ outside > Na^+ inside). The reaction was started by the addition of 20 μl of vesicles to 100 μl of incubation medium containing 100 mM-mannitol, 100 mM-NaCl, 20 mM-Hepes/Tris buffer, pH 7.4, and 50 μl of D-[^{3}H]glucose. Temperature was maintained at 37 °C. ●, K^+ gradient (K^+ outside > K^+ inside). The reaction was started by the addition of 20 μl of vesicles to 100 μl of incubation medium containing 100 mM-KCl, 100 mM-mannitol, 20 mM-Hepes/Tris buffer, pH 7.4, and 50 μl of D-[^{3}H]glucose. Temperature was maintained at 37 °C. Samples were taken at 0.3, 1, 2, 5 and 20 min. Values are means ± S.E.M. (n = 3).

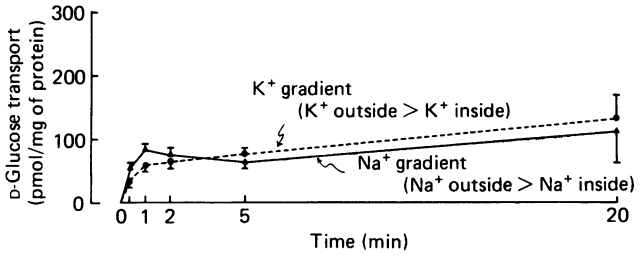

Fig. 3. D-Glucose uptake by brush-border membrane vesicles prepared from human terminal ileum

Vesicles were prepared in 200 mM-mannitol/20 mM-Hepes/Tris buffer, pH 7.4. Transport was determined under two different conditions. ▲, Na^+ gradient (Na^+ outside > Na^+ inside). The reaction was started by the addition of 20 μl of vesicles to 100 μl of incubation medium containing 100 mM-mannitol, 100 mM-NaCl, 20 mM-Hepes/Tris buffer, pH 7.4, and 50 μl of D-[^{3}H]glucose. Temperature was maintained at 37 °C. ●, K^+ gradient (K^+ outside > K^+ inside). The reaction was started by the addition of 20 μl of vesicles to 100 μl of incubation medium containing 100 mM-KCl, 100 mM-mannitol, 20 mM-Hepes/Tris buffer, pH 7.4, and 50 μl of D-[^{3}H]glucose. Temperature was maintained at 37 °C. Samples were taken at 0.3, 1, 2, 5 and 20 min. Values are means ± S.E.M. (n = 3).

functional, taurocholate uptake was determined in the presence of Na^+ and K^+ gradients. Fig. 4 depicts the uptake of [^{3}H]taurocholate from BBMV of terminal ileum. In the presence of a Na^+ gradient, taurocholate uptake at 20 s, 1, 2 and 5 min was significantly greater than corresponding values with K^+ gradient. Although equilibrium values were similar, calculation of intravesicular space for [^{3}H]taurocholate uptake indicates a volume of 4.5 ± 0.2 μl/mg of protein. These values are greater than volumes obtained for glucose (1.2 ± 0.1 μl/mg of protein). These findings are similar to previously reported volumes for [^{3}H]taurocholate and indicates significant internal binding of bile acids (Barnard *et al.*, 1985). To determine further the internal binding of bile acids, efflux experiments were conducted. Membrane vesicles were loaded with either 0.1 mM-taurocholate or 0.1 mM-D-glucose in the presence of 100 mM-NaCl, 100 mM-mannitol and 20 mM-Hepes/Tris (pH 7.4) and then incubated in 300 mM-mannitol/20 mM-Hepes/Tris (pH 7.4). Efflux was determined at 1, 2, 5 and 60 min. The amount of radioactivity remaining in the vesicles at all time points was significantly greater for [^{3}H]taurocholate than for D-glucose, indicating significant internal binding of bile acids (Fig. 5).

Tracer-exchange studies

To determine whether the difference in the magnitude of the overshoot with Na^+ gradient was secondary to changes in the Na^+–solute co-transporter activity (putative carrier), counter-transport studies were performed under conditions of Na^+ and D-glucose equilibrium. Tracer-exchange studies were selected because of the difficulties in interpreting kinetic data obtained with vesicle preparations. Because of the heterogeneity of the vesicle preparations and the changes in trans-membrane sodium and electrical gradients owing to substrate fluxes, kinetic data may not be meaningful. Tracer-exchange studies provide a constant [Na^+] and therefore are suitable alternatives for the study of kinetic parameters (Hopfer, 1978). The vesicles from jejunum, mid and terminal ileum were preincubated with unlabelled D-glucose, Na^+ and

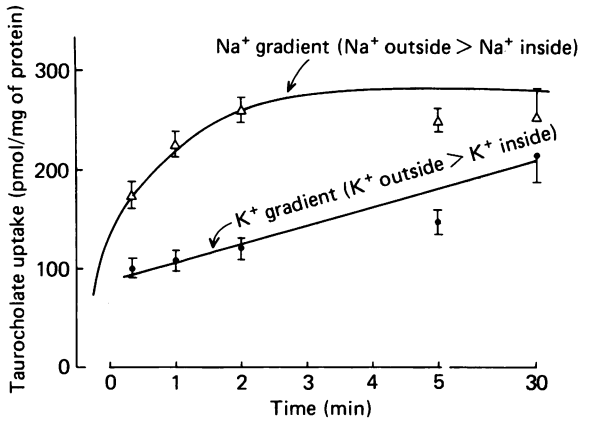

Fig. 4. Taurocholate uptake by brush-border membrane vesicles from human terminal ileum

Vesicles were prepared in 200 mM-mannitol/20 mM-Hepes/Tris buffer, pH 7.4. Transport was determined under two different conditions. ▲, Na^+ gradient (Na^+ outside > Na^+ inside). The reaction was started by the addition of 20 μl of vesicles to 100 μl of incubation medium containing 100 mM-KCl, 100 mM-mannitol, 20 mM-Hepes/Tris buffer, pH 7.4, 0.1 mM-taurocholate and tracer [^{3}H]taurocholate. ●, K^+ gradient (K^+ outside > K^+ inside). The reaction was started by the addition of 20 μl of vesicles to 100 μl of incubation medium containing 100 mM-KCl, 100 mM-mannitol, 20 mM-Hepes/Tris buffer, pH 7.4, 0.1 mM-taurocholate and tracer [^{3}H]taurocholate. Samples were taken at 0.3, 1, 2, 5 and 30 min. Values are means ± S.E.M. (n = 3).

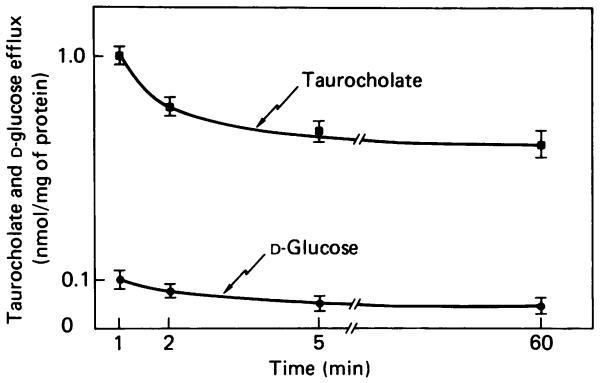

Fig. 5. [^{3}H]taurocholate and D-glucose efflux from human ileal brush-border membrane vesicles

Vesicles were preloaded with either 0.1 mM-taurocholate (■) or 0.1 mM-D-glucose (●) in 100 mM-NaCl/100 mM-mannitol/20 mM-Hepes/Tris buffer, pH 7.4, and allowed to incubate for 1 h at room temperature. Efflux was determined at 1, 2, 5 and 60 min. At all time points radioactivity remaining in the vesicles was significantly greater for [^{3}H]taurocholate than for D-glucose, indicating significant internal binding of bile acids to the inside of the vesicles.

gramicidin (gramicidin is an ionophore which increases the cation conductance of membranes and therefore nullifies the development of an electrical gradient secondary to Na^+/D-glucose efflux from the vesicles). Initial uptake in the jejunum differed significantly from that in the mid-ileum and the terminal ileum (Table 2). The change in activity indicates that the activity of glucose transporters diminishes aborally. Equilibrium values were reached only at 60 min; the number and/or activity of the glucose carriers were less in the terminal ileum. Therefore, in the absence of electrochemical potential it took more than 20 min to achieve equilibrium.

DISCUSSION

The present studies demonstrate that BBMV can be prepared from segments of human small intestine. The purity of the membranes was validated by functional criteria and was demonstrated by the enrichment of the brush-border enzyme leucine aminopeptidases in the final BBMV preparations.

The imposition of an extravesicular-to-intravesicular Na^+ gradient brought about the transient accumulation 'overshoot' of D-glucose in the jejunal and mid-ileal BBMV that was several-fold higher than that attained at equilibrium. The 'overshoot' values, however, were significantly higher in the jejunum than the mid-ileum ($P < 0.05$). The imposition of a Na^+ gradient in BBMV prepared from terminal ileum resulted in no 'overshoot' in D-glucose uptake. The imposition of a K^+ gradient resulted in no 'overshoot' accumulation of D-glucose in BBMV prepared from jejunum, mid and terminal ileum. These results indicated that the process of D-glucose uptake is Na^+-dependent in the jejunum and mid-ileum, but not in the terminal ileum. The data for jejunal D-glucose uptake are similar to those previously reported

Table 2. Regional changes in tracer-exchange experiments

BBMV from jejunum, mid-ileum and terminal ileum were preincubated for 60 min with 100 mM-mannitol, 100 mM-NaCl, 0.1 mM-D-glucose, 20 mM-Hepes/Tris buffer (pH 7.4) and 6 μg of gramicidin/ml. The reaction was started by the addition of 20 μl of preincubated vesicles to 10 ml of incubation medium containing 100 mM-mannitol, 100 mM-NaCl, 0.1 mM-D-glucose and 50 μCi of radiolabelled D-glucose. Samples were taken at 0.3, 1, 2, 5, 20 and 60 min. Values are means ± S.E.M. for four independent experiments.

	D-Glucose uptake (pmol/mg of protein)		
Time (min)	Jejunum	Mid-ileum	Terminal ileum
0.3	125 ± 10	74 ± 2	43 ± 1
1	153 ± 10	63 ± 8	51 ± 4
2	345 ± 6	78 ± 3	58 ± 2
5	303 ± 4	100 ± 50	59 ± 4
20	367 ± 15	112 ± 9	70 ± 6
60	135 ± 10	125 ± 15	128 ± 12

(Triadon *et al.*, 1983); however, to our knowledge there are no studies describing mid- and terminal-ileum D-glucose uptake at the membrane level in man. The addition of valinomycin to KCl-preincubated jejunal BBMV enhanced the Na^+-dependent transport. This supports the electrogenic nature of D-glucose transport in the proximal small intestine. The data for D-glucose transport in the mid-ileum are similar to the observations by Hopfer *et al.* (1976) for D-glucose transport by rat ileal brush-border membrane vesicles.

To determine the mechanism for the increase in the 'overshoot' in the jejunum compared with the mid-ileum, and the absence of 'overshoot' in the terminal ileum, tracer-exchange studies were performed. By using gramicidin, all electrochemical gradients across the BBMV were nullified. The resulting D-glucose uptake therefore reflected Na^+–glucose co-transporter activity. As seen in Table 2, this co-transporter activity diminished aborally. This difference in the putative carrier partially explains the greater 'overshoot' in the jejunum compared with the ileum.

The absence of the 'overshoot' phenomenon and decrease in Na^+–glucose co-transporter activity in BBMV prepared from terminal ileum indicates that this region of the small intestine plays a minimal role in overall glucose absorption in man. The data further suggest that the transport of D-glucose in the terminal ileum is non-Na^+-dependent and resembles the type of D-glucose transport present in the colon (Long *et al.*, 1967; Heaton, 1972; Wingate *et al.*, 1973).

Our studies demonstrate that transport studies using BBMV are feasible in man. We demonstrated that D-glucose uptake is Na^+-dependent and electrogenic in the proximal small intestine, which supports other studies on human D-glucose transport (Modiglinani & Bernier, 1971; Cook, 1973; Phillips & McGill, 1973; Murer & Hopfer, 1974; Hopfer *et al.*, 1975).

In our studies, D-glucose transport was minimal in the terminal ileum (near the ileocaecal valve) and did not appear Na^+-dependent. This, in part, is explained by the diminished Na^+-glucose co-transporter activity.

REFERENCES

Barnard, J. A., Ghishan, F. K. & Wilson, F. A. (1985) J. Clin. Invest. **75**, 869–873

Beaufay, H., Costesec, A. A., Feyman, E., Thines-Sempoux, T., Wibo, M., Robbie, M. & Berther, J. (1974) J. Cell Biol. **61**, 188–200

Cook, G. C. (1973) Clin. Sci. **44**, 425–430

Crane, R. K. (1962) Fed. Proc. Fed. Am. Soc. Exp. Biol. **21**, 891–895

Crane, R. K. (1965) Fed Proc. Fed. Am. Soc. Exp. Biol. **24**, 1000–1005

Evers, C., Haase, W., Murer, H. & Kinne, R. (1978) Membr. Biochem. **1**, 203–209

Ghishan, F. K. & Wilson, F. A. (1985) Am. J. Physiol. **248**, G87–G92

Heaton, J. W., Jr. (1972) Am. J. Dig. Dis. **17**, 7–16

Hopfer, U. (1978) Am. J. Physiol. **234**, F89–F96

Hopfer, U., Nelson, K., Perratto, I. & Isselbacher, K. J. (1973) J. Biol. Chem. **248**, 25–32

Hopfer, U., Sigrist-Nelson, K. & Murer, H. (1975) Ann. N.Y. Acad. Sci. **264**, 414–422

Hopfer, U., Sigrist-Nelson, K. & Groseclose, R. (1976) Biochim. Biophys. Acta **426**, 349–353

Long, C. L., Geiger, J. W. & Kinney, J. M. (1967) Metab. Clin. Exp. **16**, 413–418

Love, A. H. (1968) J. Physiol. (London) **107**, 38–39

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275

Lucke, H., Berner, W., Menge, H. & Murer, H. (1978) Pfluegers Arch. **373**, 242–248

McLaughlin, S. & Eisenberg, M. (1975) Annu. Res. Biophys. Bioeng. **4**, 335–362

Modiglinani, R. & Bernier, J. J. (1971) Gut **12**, 184–190

Murer, H. & Hopfer, U. (1974) Proc. Natl. Acad. Sci. U.S.A. **71**, 484–488

Murer, H. & Kinne, R. (1980) J. Membr. Biol. **55**, 81–95

Murer, H., Hopfer, U., Kinne-Saffran, E. & Kinne, R. (1974) Biochim. Biophys. Acta **345**, 170–179

Phillips, S. F. & McGill, D. B. (1973) Am. J. Dig. Dis. **18**, 1017–1023

Triadon, N., Bataille, J. & Schmitz, J. (1983) Gastroenterology **85**, 1326–1332

Wilson, F. A. & Treanor, L. (1981) Gastroenterology **81**, 54–66

Wimberly, P. D., Harris, T. J. & Burgers, E. A. (1973) Proc. Soc. Exp. Biol. Med. **67**, 755–759

Wingate, D. L., Krag, E., Mekhjian, H. S. & Phillips, S. F. (1973) Clin. Sci. **45**, 593–606

Received 25 November 1985/19 February 1986; accepted 7 March 1986