Sodium Ion/L-Lactate Co-Transport in Rabbit Small-Intestinal Brush-Border-Membrane Vesicles

Bruno HILDMANN, Carlo STORELLI,* Winfried HAASE, Mario BARAC-NIETO† and Heini MURER

Max-Planck-Institut für Biophysik, Kennedyallee 70, 6000 Frankfurt 70, Germany

(Received 15 May 1979)

Uptake of L-lactate into rabbit jejunal brush-border-membrane vesicles prepared by a Ca^{2+} -precipitation procedure was studied by a rapid filtration technique with L-[^{14}C]-lactate as tracer. Transport of L-lactate into an intravesicular (osmotically reactive) space could be established. An inwardly directed NaCl gradient (outside 21 mM/inside 0 mM) stimulated the uptake of L-lactate at 15 s 2–4-fold compared with that observed with an equal KCl gradient. A transient accumulation of L-lactate inside the vesicles (overshoot) was observed in the presence of an NaCl gradient. Gradients of LiCl, RbCl, CsCl or choline chloride were not able to replace NaCl in the stimulation of L-lactate uptake. L-Lactate uptake was saturable only in the presence of Na^+ . D-Lactate, DL-thio-lactate (2-DL-mercaptopropionate), pyruvate and propionate inhibited the Na^+ -stimulated L-lactate uptake; D-lactate, thiolactate and pyruvate provoked trans-stimulation of L-lactate uptake. Artificially imposed diffusion potentials (inside negative) did not exert any effect on the Na^+ -dependent L-lactate uptake. The results are consistent with the existence of an electroneutral $\text{Na}^+/\text{L-lactate}$ co-transport system in the brush border of rabbit small intestine.

The data presently available do not allow us to draw a conclusive picture of how lactic acid, which under physiological pH conditions is present in its anionic form, is transported in the small intestine. Studies on the steady-state metabolism and transport of D-glucose using everted-sac preparations of rat jejunum have indicated that the intracellular lactate concentration is higher than predicted from equilibrium distribution (Pritchard & Porteous, 1977). Consequently, one could speculate that the luminal membrane of the intestinal epithelial cell contains system(s) that at least counteract the diffusional loss of lactate produced intracellularly by the glycolytic pathway of the cell. Furthermore, it might possess a system able to transport lactate present in the intestinal lumen against its concentration gradient into the cell. By analogy with the transport of sugars and amino acids (Hopfer, 1978), Na^+ might play a role in the transport across the brush-border membrane. In a study with brush-border membranes isolated from rat kidney cortex, it has been demonstrated that lactate transport across this

membrane proceeds via a $\text{Na}^+/\text{lactate}$ co-transport system (Barac-Nieto *et al.*, 1979). A similar approach with the use of isolated brush-border membranes from rabbit small intestine was undertaken in the present study. The results indicate that electroneutral $\text{Na}^+/\text{L-lactate}$ co-transport is involved in the transfer of lactate across rabbit intestinal brush-border membranes.

A poster of this study has been given at the Joint Meeting of the Biochemical Society and the Gesellschaft für Biologische Chemie (Hildmann *et al.*, 1978).

Methods

Preparation of brush-border-membrane vesicles

Brush-border-membrane vesicles were isolated from fresh rabbit jejunum. A Ca^{2+} -precipitation procedure modified from the methods originally described by Schmitz *et al.* (1973) for human intestine and Kessler *et al.* (1978) for frozen rabbit intestine was applied, which was similar to that described by Lücke *et al.* (1978a) for the preparation of human intestinal brush-border-membrane vesicles. Rabbits were killed by a blow on the neck. The jejunum was quickly removed and immersed in ice-

* On leave from the Università degli Studi di Bari, Bari, Italy.

† On leave from the Universidad del Valle, Cali, Colombia.

cold Ringer's solution. Then it was rinsed with Ringer's solution and everted. The mucosa was scraped off with a slide. The scrapings were suspended in 300 mM-mannitol/12 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]/Tris, pH 7.1 (60 ml/10 g of mucosa). The suspension was diluted 6:1 with water and immediately homogenized in portions not exceeding 300 ml for 3 min at maximal speed in a blender (Braun, Kronberg, Germany). After addition of CaCl_2 to a final concentration of 10 mM the homogenate was processed as described by Lücke *et al.* (1978a), with the following modifications: for the first two centrifugations, the Sorvall GSA rotor was used at centrifugal forces equivalent to that given by Lücke *et al.* (1978a); pellet 2 and pellet 4 were resuspended in 300 mM-mannitol/20 mM-Hepes/Tris, pH 7.4 (40 ml of buffer/10 g of mucosa and 1 ml of buffer/10 g of mucosa respectively). Membrane suspensions used for the uptake studies had a protein concentration of about 10 mg/ml. Preloading of the vesicles with NaCl or KCl was carried out by the addition of substances to the membrane suspension and preincubation for 1 h in the cold. Preloading with structural analogues of L-lactate was done by resuspending pellet 2 and the subsequent pellets in buffers containing these substances at the desired concentration.

Enzyme assays

Alkaline phosphatase (EC 3.1.3.1) and $(\text{Na}^+ + \text{K}^+)\text{-stimulated ATPase}$ (EC 3.6.1.3) were measured as described by Berner & Kinne (1976). Lactate dehydrogenase (EC 1.1.1.27) was measured by using the Merckotest no. 3367. NADH-(acceptor) oxidoreductase (EC 1.6.99.3) was measured as described by Wallach & Kamat (1966), with the addition of KCN as described by Sottocasa *et al.* (1967). Succinate-cytochrome *c* oxidoreductase (EC 1.3.99.1) was measured as described by Fleischer & Fleischer (1967). Acid phosphatase (EC 3.1.3.2) was measured by the method of Shapiro (1979). Protein was determined by the method of Lowry *et al.* (1951), after precipitation with ice-cold 10% (w/v) trichloroacetic acid, with bovine serum albumin as standard.

Uptake measurements

Solute uptake was measured by using radioactively labelled substrates: L-[U- ^{14}C]lactate (sodium salt; 138.6 mCi/mmol; NEC-599; NEN Chemicals G.m.b.H., Dreieich, Germany) and D-[U- ^{14}C]glucose (313.0 mCi/mmol; NEC-042x; NEN Chemicals G.m.b.H.); 100 μl of incubation medium contained 5 μCi of L-[U- ^{14}C]lactate or 5 μCi of D-[U- ^{14}C]glucose. Membranes were usually suspended in 300 mM-mannitol/20 mM-Hepes, adjusted to pH 7.4 with Tris. Exceptions are indicated in the legends to

the Figures and Tables. Incubation media contained, unless otherwise stated in the legends, 300 mM-mannitol, 20 mM-Hepes/Tris, pH 7.4, and 0.3–0.55 mM-L-lactate; additions of salts or inhibitors are given in the legends. Incubations were started by adding 10–40 μl of membrane suspension to 80–240 μl of incubation medium kept at 25°C. The uptake was stopped by pipetting a portion (20 μl) of the reaction mixture on to the middle of a membrane filter (cellulose nitrate; 0.6 μm pore size; Sartorius, Göttingen, Germany) kept under suction. The filter was immediately washed with 5 ml of ice-cold solution containing 330 mM-mannitol, 20 mM-Hepes/Tris, 28 mM-NaCl and 0.2 mM- HgCl_2 , and also 0.2 mM-phlorrhizin, when D-glucose uptake was studied. The efficiency of the washing was controlled as follows: 20 μl of membrane suspension of the same protein content as the incubation media was put on the filters, and then after 1 s 20 μl of incubation medium without membrane was pipetted on to the same spot, and the filter washed as usual. For up to 60 μg of protein per filter no increase in non-specific retention of radioactivity on the filters could be detected compared with blanks consisting of membrane-free incubation medium. The radioactivity remaining on the filters was analysed by standard liquid-scintillation counting techniques. Rotiszint 33 (Roth, Karlsruhe, Germany) was used as scintillator, which completely solubilized the radioactivity from the filters. All experiments were performed in duplicate. In general, representative experiments are presented. All types of experiments were performed at least three times with qualitatively the same results. If statistical evaluation of small effects was necessary, several experiments were averaged by standardizing the uptake results and expressing the uptake at different time points as a percentage of the equilibrium value, in order to avoid difficulties with the variation in the equilibrium values found for the different preparations.

T.l.c.

Samples were taken from the incubation media after 1 h and put into an excess volume of the eluent used for chromatographic separation; alternatively they were filtered as in the uptake experiments and the filters extracted with the eluent. The samples were then concentrated by the use of a rotating evaporator, and chromatographed on precoated silica gels with the following eluent mixture: ethanol/chloroform/ NH_3 /water (530:303:152:15, by vol.; Copius-Peereboom, 1967). Efficiency of separation was controlled with L-lactate, pyruvate, thiolactate (2-DL-mercaptopyruvate), L-alanine and propionate. The chromatograms were stained in an iodine chamber. For liquid-scintillation counting the chromatograms were cut into strips and counted for radioactivity in a similar way to the

uptake samples. More than 97% of the radioactivity present in the incubation medium was found to be associated with lactate after 1 h of incubation. At least 90% of the radioactivity taken up by the vesicles was recovered as lactate.

Electron microscopy

Brush-border membrane fractions were fixed in suspension with 2.5% (w/v) glutaraldehyde in 0.1 M-sodium cacodylate buffer (pH 7.4) for 2 h. After being washed in cacodylate buffer they were treated with 1% OsO_4 in 0.05 M-veronal/acetate buffer (pH 7.2), and washed in water afterwards. The material was stained for 1 h with 2% uranyl acetate, washed with water, dehydrated and embedded in low-viscosity resin (Spurr, 1969). Thin sectioning was performed with an LKB ultramicrotome. The sections were stained with lead citrate and examined in a Philips EM 300 electron microscope.

For negative staining a droplet of the material was applied to a Formvar- and carbon-coated grid, and stained with 5% ammonium molybdate (pH 5.0).

Materials

Tris, mannitol, ouabain, thiolactic acid (2-DL-mercaptpropionic acid), sodium succinate, cytochrome *c*, potassium ferricyanide and NADH were obtained from Merck, Darmstadt, Germany. Hepes, D-lactate (lithium salt), L-lactate (lithium salt), D-glucose and cellobiose came from Serva, Heidelberg, Germany. Pyruvic acid was purchased from Sigma, Munich, Germany. Valinomycin, NADP^+ , ATP, hexokinase and glucose 6-phosphate dehydrogenase were obtained from Boehringer, Mannheim, Germany.

Results and Discussion

Characterization of the brush-border-membrane fraction

Enzyme content. The purity of the brush-border-membrane fraction was controlled by the assay of alkaline phosphatase, a commonly used marker enzyme for brush-border membranes. The enrichment of alkaline phosphatase over that in the homogenate was 26-fold $\pm$ 3.4-fold (mean $\pm$ S.E.M., $n = 17$). In some preparations other marker enzymes were measured to establish which contaminants were present. For NADH-(acceptor) oxidoreductase, succinate-cytochrome *c* oxidoreductase and $(\text{Na}^+ + \text{K}^+)$ -stimulated ATPase a lower specific activity was found in the membrane fraction, whereas the acid phosphatase was enriched by a factor of 1–3. In agreement with the findings of Evers *et al.* (1978) for the preparation of kidney-cortical brush-border membranes by the Ca^{2+} -precipitation method, we conclude that the fraction is contaminated by lysosomes.

Morphology. Morphological characterization of the brush-border-membrane fraction was carried out by electron microscopy. Examination of thin sections revealed that the fraction is dominated by structures of brush-border origin (e.g. Plate 1), characteristics of which are the electron-dense contents enclosed by the membranes and fuzzy structures covering the outer surfaces of the membranes (for a detailed view, see Plate 1b). Contaminating membranes, not exhibiting the above characteristics, are rarely seen. The brush-border membranes appear in two forms: (a) ellipsoidal vesicles of 80–280 nm diameter range (marked with asterisks in Plate 1b); (b) rod-shaped fragments of microvilli. The latter display typical filamentous structures inside (Plate 1b, arrows). These two main morphological groups in the brush-border-membrane fraction are confirmed by their appearance in negative-staining preparations (Plate 2). Whether both groups of structures derived from the luminal cell surface contribute to the intravesicular space cannot be decided. However, from the inhomogeneity of the membrane fraction it is evident that variations in the relative portions of the two main constituents from one preparation to the other, which have actually been observed, could explain the variations in the equilibrium uptake of L-lactate mentioned above, since this leads to variations in volume/surface and hence volume/protein ratios.

Transport studies

Effect of a Na^+ gradient on the uptake of L-lactate. Jejunal brush-border membranes were prepared in a Na^+ - and K^+ -free buffer and subsequently incubated in an NaCl- or a KCl-containing medium (Fig. 1). With the Na^+ gradient the initial uptake of L-lactate is faster than with the K^+ gradient; the ratio of the uptakes after 15 s of incubation is 2–4. The Na^+ -gradient-stimulated uptake after 1 min of incubation exceeds the equilibrium uptake (reached

Fig. 1. Effect of Na^+ on L-lactate uptake by brush-border vesicles isolated from rabbit jejunum

Membrane suspension (40 μl) was added to 240 μl of incubation medium containing 25 mM-NaCl (●) or -KCl (○). The final concentration of L-lactate was 0.54 mM.

after 20 min) by 30–100%. This overshoot phenomenon implies a transient accumulation of L-lactate inside the vesicles. The energy for the transient uphill transport of L-lactate is thought to be delivered by the dissipating Na^+ gradient via a flux-coupling mechanism, as it has been worked out for other substrates (Hopfer *et al.*, 1973; Murer & Kinne, 1977; Hopfer, 1978). A slightly higher equilibrium uptake in the K^+ -containing medium than in the Na^+ -containing medium was seen in a number of experiments. Possible explanations for this observation would be that incubation with K^+ slightly increases the degree of vesiculation or that the amount of binding of L-lactate to the membranes is increased (for binding see below). Since the differences in the equilibrium uptakes observed with Na^+ and K^+ were small compared with the Na^+ -specific effects during the early incubation period, no attempts were made to define the nature of the differences in equilibrium uptakes.

Uptake into the intravesicular space. Uptake of a solute into vesicular membraneous structures may represent binding to the membrane or entrance into the intravesicular space. In order to establish that transport of L-lactate into an intravesicular space takes place, the vesicles were prepared in a buffer containing 100 mM-cellobiose instead of mannitol, and the osmolarity of the incubation medium was increased stepwise by adding increasing amounts of cellobiose. These conditions should lead to a shrinkage of the vesicles during the incubation time. This behaviour has been demonstrated, e.g. for rat intestinal brush-border vesicles in investigations of transport of D-glucose and L-alanine and for rabbit intestinal brush-border vesicles in a study of D-glucose transport (Hopfer *et al.*, 1973; Sigrist-Nelson *et al.*, 1975; Kessler *et al.*, 1978). The data reported in these papers support the notion that the isolated brush-border-membrane vesicles behave like osmometers in media in which cellobiose makes up the major portion of the solutes, i.e. the permeability of the brush-border membranes to cellobiose is low compared with that to water. Fig. 2 shows the dependence of the equilibrium uptake of L-lactate and D-glucose on the reciprocal of the cellobiose concentration in the incubation medium. Different ordinates are given for the two substrates, because L-lactate was present at a 2.26-fold higher concentration than D-glucose. Both substances seem to enter an intravesicular space, since the equilibrium up-

Fig. 2. Influence of osmolarity of the medium on the equilibrium uptake of L-lactate and D-glucose in intestinal brush-border-membrane vesicles

Membrane vesicles were prepared by using 100 mM-cellobiose instead of mannitol throughout the preparation. Incubation media and membrane suspensions contained 2 mM-Hepes/Tris (pH 7.4). Incubation media contained 25 mM-NaCl and 100 mM-, 125 mM-, 167 mM-, 250 mM- or 500 mM-cellobiose. The uptake was initiated by the addition of 20 μl of membrane suspension to 80 μl of incubation medium, leading to concentrations of 100 mM-, 120 mM-, 154 mM-, 220 mM- or 420 mM-cellobiose outside the vesicles. The final substrate concentrations were 0.43 mM-L-lactate (●) and 0.19 mM-D-glucose (○).

takes decrease with increasing osmolarity of the medium. Furthermore, they seem to enter the same intravesicular space, since equilibrium uptakes of D-glucose and L-lactate decrease in a parallel manner. When the lines in Fig. 2 are extrapolated to infinite osmolarity of the medium, the portion of the uptake that appears to be independent of the intravesicular volume is obtained as the intercept on the ordinate. This might represent binding to some component of the vesicular membrane or transport into a compartment that is not available to further osmotic shrinkage. The latter explanation is more likely to correspond to our experimental observation, since the uptake of two different solutes, namely D-glucose and L-lactate, show a similar osmotic dependence. In earlier studies, in which intestinal brush-border membranes obtained by different methods were used, it has been demonstrated that no binding of D-glucose to brush-border membranes can be detected (Hopfer *et al.*, 1973; Murer *et al.*, 1974). Therefore we postulate that vesicular shrinkage due

EXPLANATION OF PLATE 1

Thin section of a brush-border-membrane fraction

(a) Most of the membrane vesicles contain electron-dense material. They have either retained their rod shape or formed ellipsoidal vesicles of 80–280 nm mean diameter. Magnification $\times 20000$. (b) The fuzzy coat typical of brush-border membranes can be clearly seen (surface of vesicles marked by asterisks). The rod-shaped vesicles display filamentous material inside (arrows). Magnification $\times 66000$.

EXPLANATION OF PLATE 2

Negative-staining preparation of a brush-border membrane fraction

The shape of the vesicles varied from large longitudinal ones to smaller irregularly shaped ones as well as almost spherical ones (asterisks). Their surfaces display knob-like structures (arrows). Magnification $\times 66\,000$.

Table 1. *Uptake of L-lactate into brush-border-membrane vesicles of small intestine with gradients of different cations*

Membrane suspension (20 μ l) was added to 120 μ l of incubation medium containing 25 mM-chloride salts. The final concentration of L-lactate was 0.36 mM. The uptake is given in pmol/mg of protein.

	L-Lactate uptake			
	15 s	1 min	2 min	Equilibrium
LiCl	147	244	277	369
KCl	212	293	338	450
RbCl	230	347	328	426
CsCl	336	207	393	517
Choline chloride	256	231	314	413
NaCl	529	737	478	392

to osmolarity is hindered in our membrane fraction prepared by Ca^{2+} precipitation. From the electron micrographs (Plates 1a and 1b), this seems to be reasonable, since a large portion of the vesicles are filled by electron-dense material originating from the core of the microvilli. As far as measuring transport is concerned, it may be concluded that at least two-thirds of the uptake measured represents transport.

Cation specificity. The uptake of L-lactate with gradients of different chloride salts of univalent cations is given in Table 1. The values at 15 s obtained with Li^+ , K^+ , Rb^+ , Cs^+ and choline are small compared with those obtained with Na^+ , and only with Na^+ is an overshoot observed. Such a strict Na^+ specificity has also been observed for the intestinal Na^+ /taurocholate co-transport (Lücke *et al.*, 1978b), whereas the intestinal Na^+ -dependent transport systems for amino acids (Sigrist-Nelson *et al.*, 1975) and P_i (Berner *et al.*, 1976) were also driven to a significant extent by Li^+ .

Indirect or direct coupling of L-lactate- and Na^+ fluxes. In order to answer the question of whether Na^+ and L-lactate fluxes are coupled by a diffusion potential caused by the dissipating NaCl gradient driving the L-lactate anion (only 1/4000th of the lactic acid molecules are in the undissociated form at pH 7.4) across the membrane, valinomycin-treated membranes were incubated in a KCl-containing medium (Table 2). These conditions are known to create an inside-positive diffusion potential compared with the control without valinomycin, since valinomycin selectively increases the K^+ permeability of the membrane (Pressmann, 1968). No stimulation of the L-lactate influx by a K^+ gradient could be observed under these conditions, whereas the Na^+ gradient leads to a transient accumulation of L-lactate inside the vesicles. Therefore a diffusion potential does not seem to be involved in the coupling of Na^+ and L-lactate fluxes. As a Na^+ / H^+ -exchange system is present in the brush-order mem-

Table 2. *Effect of an inside-positive diffusion potential on the L-lactate uptake into rabbit jejunal brush-border-membrane vesicles*

Membrane vesicles were prepared in 300 mM-mannitol/20 mM-Hepes/Tris (pH 7.4): 2 μ l of valinomycin in ethanol (10 mg/ml) was added to 100 μ l of membrane suspension for condition II; the same amounts of ethanol (2% final concentration) were added to the membranes for conditions I and III; 20 μ l of membrane suspension was then added to 120 μ l of incubation medium containing 25 mM-NaCl or 25 mM-KCl. The final concentration of L-lactate was 0.36 mM. The uptake is expressed as pmol/mg of protein.

Time of uptake	L-Lactate uptake		
	I KCl	II KCl + valinomycin	III NaCl
15 s	121	132	247
1 min	170	151	351
2 min	205	190	321
4 min	196	195	302
1 h	285	293	290

Table 3. *Uptake of L-lactate into NaCl- or KCl-pre-equilibrated brush-border-membrane vesicles of rabbit jejunum*

Vesicles were preloaded with 25 mM-NaCl or -KCl; 10 μ l of membrane suspension was added to 110 μ l of incubation medium containing 25 mM-NaCl or -KCl respectively. The final concentration of L-lactate was 0.5 mM. The uptake is given as pmol/mg of protein.

Time of uptake	L-Lactate uptake	
	Na^+ -equilibrated	K^+ -equilibrated
15 s	100	45
35 s	131	63
2 h	138	134

brane of rat intestine (Murer *et al.*, 1975) and rabbit intestine (H. Murer & R. Kinne, unpublished observations), the dissociation equilibrium of L-lactate and the free lactic acid might be shifted to the free acid by a Na^+ -stimulated acidification of the outside medium. The uncharged form might then be able to enter the vesicles through the hydrophobic barrier of the membrane.

In order to prevent such an effect we used a high buffering capacity (20 mM-Hepes/Tris) throughout the experiments, which had been shown by establishing a titration curve (not shown) to be sufficient to prevent significant pH changes. Assuming a one per one exchange of Na^+ and H^+ and 1% intravesicular volume compared with the total volume, the maximal pH difference to be expected in the extravascular space is 0.1, which cannot cause a significant increase in free lactic acid concentration at

a pH that is 3.6 pH units from the pK of 3.8. Positive experimental evidence for direct coupling of Na^+ and L-lactate fluxes was obtained from experiments in which L-lactate uptake was measured with equal concentrations of Na^+ or K^+ on both sides of the membranes. As shown in Table 3, L-lactate equilibrates much faster across the membrane with Na^+ than with K^+ . Under these conditions the only driving force for the influx of L-lactate is the electrochemical potential difference of L-lactate. So the result is best explained by a facilitated diffusion mechanism working only, or at least preferentially, with Na^+ present. Further evidence for direct coupling of L-lactate and Na^+ fluxes at distinct sites is saturability of L-lactate uptake only in the presence of Na^+ , and the trans-stimulation by L-lactate and some structural analogues, as shown below.

Having previously excluded significant amounts of indirect coupling via diffusion potentials or pH gradients, we may be quite sure, that there is also

Fig. 3. Dependence of L-lactate uptake on substrate concentration

Membrane suspension (20 μl) was added to 80 μl of incubation medium containing 25 mM-NaCl (●) or 25 mM-KCl (▲) and increasing concentrations of L-lactate to give 0.35, 0.7, 2.1, 6.3 and 12.6 mM final concentrations. The Na^+ -specific uptake was calculated by subtracting the uptake with KCl from that with NaCl.

Table 4. Effects of structural analogues of L-lactate on the Na^+ -dependent L-lactate uptake into rabbit jejunal brush-border-membrane vesicles

(a) Cis-inhibition and (b) trans-stimulation: 10 μl of membrane suspension was added to 110 μl of incubation medium containing 25 mM-NaCl. (a) Concentrations of L-lactate and inhibitors in the reaction mixture were 0.5 mM on the outside of the membranes at the start of the incubation. (b) Membrane vesicles were preloaded with 6 mM structural analogue, which resulted in a gradient across the membrane (6 mM inside/0.5 mM outside) at the start of the incubation. The final concentration of L-lactate was 0.5 mM. The significance of differences between inhibited or trans-stimulated uptakes and controls was tested by the t test for paired data. The uptake is given as percentage of equilibrium uptake (mean $\pm$ S.E.M.)

(a)						
Substance tested for cis-inhibition	Incubation time (s)	<i>n</i>	Control without inhibitor (A)	Sample + inhibitor (B)	<i>A - B</i>	<i>P</i>
D-Lactate	15	5	107.4 $\pm$ 18.3	65.7 $\pm$ 5.2	41.8 $\pm$ 15.9	0.1
	35	8	138.3 $\pm$ 11.9	99.5 $\pm$ 7.3	38.8 $\pm$ 7.2	0.0025
DL-Thiolactate	15	6	114.2 $\pm$ 16.4	77.9 $\pm$ 9.6	36.3 $\pm$ 11.9	0.1
	35	8	138.3 $\pm$ 11.9	97.3 $\pm$ 8.6	41.0 $\pm$ 6.9	0.005
Pyruvate	15	7	99.6 $\pm$ 15.2	72.5 $\pm$ 9.6	27.1 $\pm$ 7.4	0.01
	35	8	134.2 $\pm$ 11.5	105.8 $\pm$ 14.3	28.4 $\pm$ 8.5	0.01
Propionate	15	7	83.0 $\pm$ 13.0	69.4 $\pm$ 9.7	13.6 $\pm$ 7.1	0.1
	35	7	118.8 $\pm$ 10.7	96.5 $\pm$ 11.0	23.3 $\pm$ 10.8	0.05
(b)						
Substance tested for trans-stimulation	Incubation time (s)	<i>n</i>	Control without trans-stimulating agent (C)	Sample + trans-stimulating agent (D)	<i>D - C</i>	<i>P</i>
D-Lactate	15	4	80.5 $\pm$ 5.4	102.8 $\pm$ 12.2	22.3 $\pm$ 8.2	0.05
	35	4	103.1 $\pm$ 6.5	120.3 $\pm$ 8.3	17.1 $\pm$ 7.3	0.05
DL-Thiolactate	15	4	80.5 $\pm$ 5.4	81.3 $\pm$ 6.4	0.8 $\pm$ 9.9	—
	35	4	103.1 $\pm$ 6.5	116.3 $\pm$ 4.3	13.1 $\pm$ 4.1	0.025
Pyruvate	15	4	80.5 $\pm$ 5.4	105.0 $\pm$ 9.8	24.5 $\pm$ 7.6	0.025
	35	4	103.1 $\pm$ 6.5	123.8 $\pm$ 12.5	20.6 $\pm$ 12.7	0.15
L-Lactate	15	4	80.5 $\pm$ 5.4	112.3 $\pm$ 4.6	31.8 $\pm$ 3.8	0.0025
	35	4	103.1 $\pm$ 6.5	139.0 $\pm$ 17.4	35.9 $\pm$ 11.2	0.025

direct coupling of Na^+ and L-lactate fluxes in the Na^+ -gradient experiments.

Saturation of Na^+ -dependent L-lactate uptake. The initial uptake of L-lactate stimulated by a Na^+ gradient was measured with different L-lactate concentrations in the incubation medium. Fig. 3 shows the results of such an experiment. In the presence of a K^+ gradient the L-lactate uptake after 30 s is a linear function of the substrate concentration. With a Na^+ gradient a saturable component apparently adds to the unsaturable one. Saturation is expected for processes in which a limited number of distinct sites is involved. Thus the fact that L-lactate uptake has a saturable component in the presence of Na^+ is further evidence that Na^+ activates a membrane component that is able to enhance L-lactate transport across the brush-border membrane.

Substrate specificity. The question of whether structural analogues of L-lactate are able to interact with the co-transport system has been approached by two types of experiment. 1. *Cis-inhibition.* In these experiments the substance in question was present together with L-lactate outside the vesicle (*cis* side).

Fig. 4. Effect of an inside-negative diffusion potential on the Na^+ /L-lactate co-transport in intestinal brush-border-membrane vesicles

Membranes were loaded with 25 mM-KCl and 25 mM-NaCl. Valinomycin dissolved in ethanol was added to the membranes to a final concentration of 20 $\mu\text{g}/\text{mg}$ of protein. The final ethanol concentration in the membrane suspensions (also controls) was 2%. Incubation media contained 25 mM-NaCl and 50 mM-D-mannitol for osmotic compensation. ●, D-Glucose + valinomycin; ○, D-glucose without valinomycin; ■, L-lactate + valinomycin; □, L-lactate without valinomycin. The final substrate concentrations were 0.32 mM-L-lactate and 0.14 mM-D-glucose.

An inhibitory effect is expected, when L-lactate and its structural analogue are both accepted by the co-transport system. 2. *Trans-stimulation.* The structural analogue is preloaded into the membranes, i.e. it is present in the vesicle interior (*trans* side), when the incubation is started; labelled L-lactate (transported substrate) is present initially only in the incubation medium at the outside of the membrane. Under these conditions, stimulation of L-lactate uptake is expected if the structural analogue is also translocated via the same co-transport mechanism. The results of these experiments are given in Table 4. The inhibition experiments revealed that D-lactate, DL-thiolactate, pyruvate and propionate decrease L-lactate uptake significantly. In the trans-stimulation experiments D-lactate and pyruvate have significant stimulatory effects. Also, DL-thiolactate significantly increases the uptake of L-lactate at the 35 s time point, whereas it has no effect within 15 s. The effects of propionate remain below the level of significance, though a tendency to trans-stimulation could be seen (results not shown). Taking together the *cis*-inhibition and trans-stimulation experiments, there is good evidence that D-lactate and pyruvate can cross the membrane by the same transport system as L-lactate. Whether DL-thiolactate and propionate also share the transport system cannot be decided, although the results suggest some interaction.

Rheogenicity. The question of whether the Na^+ /L-lactate co-transport is influenced by the membrane potential (rheogenicity) has been elucidated by experiments that had been successfully used for Na^+ /D-glucose co-transport (Murer & Hopfer, 1974) and Na^+ /L-alanine co-transport (Sigrist-Nelson *et al.*, 1975). An inside-negative diffusion potential was created across the brush-border membranes by treating K^+ -preloaded vesicles with valinomycin and incubating them in a K^+ -free medium.

Under these conditions an inside-negative diffusion potential is generated as compared with the control without valinomycin.

Fig. 4 shows such an experiment in which KCl was used for preloading the membrane vesicles. The L-lactate uptake does not respond to the potential imposed, whereas under the same conditions D-glucose uptake is stimulated to a more than 10-fold overshoot. Similar results were also obtained with Na^+ concentrations up to 100 mM under gradient and non-gradient conditions (results not shown). It is concluded therefore that Na^+ /L-lactate co-transport across rabbit jejunal brush-border membranes occurs electroneutrally.

Conclusions

The experiments reported above are consistent

with the existence of a Na^+ /lactate co-transport system in the brush-border membrane of rabbit jejunum. The phenomenon of Na^+ stimulation was also observed in ileal-membrane preparations, but not in duodenal membranes (results not shown). In the course of our experiments special attention was paid to the mode of coupling between Na^+ flux and lactate flux. As discussed above, the observed stimulation of lactate flux by Na^+ is the consequence of a Na^+ /lactate co-transport system rather than the consequence of indirect coupling phenomena such as diffusion potentials or pH effects. In contrast with the analogous system of the rat renal tubular brush-border membrane (Barac-Nieto *et al.*, 1979), the system of the rabbit small-intestinal brush-border membrane does not seem to be influenced by alterations in the transmembrane electrical-potential difference. This observation implies that the chemical instead of the electrochemical potential difference of Na^+ is the major driving force for lactate transport across the intestinal brush-border membrane.

Our results provide limited information on the substrate specificity of the Na^+ /L-lactate co-transport system. D-Lactate and pyruvate seem also to be accepted and transported by the system, but quantitative estimations of relative affinities are not possible. In particular, the interesting question of whether short-chain fatty acids share the transport system (Naupert, 1974; Lamers, 1977) has not been elucidated.

For further insight into the handling of lactate by the intestinal epithelial cell, knowledge about the pathway for lactate transport in the contraluminal membrane is required. Further investigations with basolateral plasma membranes and brush-border membranes from rat small-intestinal epithelial cells show that there is a polar distribution of different lactate-transport systems between the two sides of the intestinal epithelial cell: a Na^+ -dependent transport system in the brush-border membrane and a Na^+ -independent facilitated diffusion system in the basolateral membrane (C. Storelli, A. Corcelli, B. Hildmann, H. Murer & C. Lippe, unpublished work). Such an arrangement of different transport systems, one of which is energized by the Na^+ gradient, would be sufficient to explain transepithelial transport of lactate from the intestinal lumen to the interstitial fluid, as it has been discussed by Murer & Kinne (1977).

We thank Professor K. J. Ullrich for valuable discussions during the preparation of the manuscript, Mrs. A. Schmidt for skilful technical assistance, and Mrs. I.

Rentel and Mrs. U. Silz-Riebandt for the excellent production of the Figures.

References

- Barac-Nieto, M., Kinne, R. & Murer, H. (1979) *Am. J. Physiol.* in the press
- Berner, W. & Kinne, R. (1976) *Pflügers Arch.* **361**, 269–277
- Berner, W., Kinne, R. & Murer, H. (1976) *Biochem. J.* **160**, 467–474
- Copius-Peereboom, J. W. (1967) in *Dünnschicht-chromatographie* (Stahl, E., ed.), 2nd edn., pp. 620–624. Springer-Verlag, Berlin, Heidelberg and New York
- Evers, C., Haase, W., Murer, H. & Kinne, R. (1978) *Membr. Biochem.* **1**, 203–219
- Fleischer, S. & Fleischer, B. (1967) *Methods Enzymol.* **10**, 406–432
- Hildmann, B., Lücke, H., Barac-Nieto, M. & Murer, H. (1978) *Hoppe-Seyler's Z. Physiol. Chem.* **359**, 1097–1098
- Hopfer, U. (1978) *Am. J. Physiol.* **234**, F89–F96
- Hopfer, U., Nelson, K., Perrotto, J. & Isselbacher, K. J. (1973) *J. Biol. Chem.* **248**, 25–32
- Kessler, M., Acuto, O., Storelli, C., Murer, H., Müller, M. & Semenza, G. (1978) *Biochim. Biophys. Acta* **506**, 136–154
- Lamers, J. M. J. (1977) in *Intestinal Permeation* (Kramer, M. & Lauterbach, F., eds.), pp. 164–172. Excerpta Medica, Amsterdam and Oxford
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Lücke, H., Berner, W., Menge, H. & Murer, H. (1978a) *Pflügers Arch.* **373**, 243–248
- Lücke, H., Stange, G., Kinne, R. & Murer, H. (1978b) *Biochem. J.* **174**, 951–958
- Murer, H. & Hopfer, U. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 484–488
- Murer, H. & Kinne, R. (1977) *FEBS Symp.* **42**, 292–302
- Murer, H., Hopfer, U., Kinne-Saffran, E. & Kinne, R. (1974) *Biochim. Biophys. Acta* **345**, 170–179
- Murer, H., Hopfer, U. & Kinne, R. (1975) *Biochem. J.* **154**, 597–604
- Naupert, Ch. (1974) Ph.D. Thesis, University of Ulm
- Pressmann, B. (1968) *Fed. Proc. Am. Soc. Exp. Biol.* **27**, 1283–1288
- Pritchard, P. J. & Porteous, J. W. (1977) *Biochem. J.* **164**, 1–14
- Schmitz, J., Preiser, H., Maestracci, D., Ghosh, B. K., Cerda, J. J. & Crane, R. K. (1973) *Biochim. Biophys. Acta* **323**, 98–112
- Shapiro, M. (1979) Ph.D. Thesis, University of Frankfurt
- Sigrist-Nelson, K., Murer, H. & Hopfer, U. (1975) *J. Biol. Chem.* **250**, 5674–5680
- Sottocasa, G. L., Kuylenstierna, B., Ernster, L. & Bergstrand, A. (1967) *J. Cell Biol.* **32**, 415–438
- Spurr, A. R. (1969) *J. Ultrastruct. Res.* **26**, 31–43
- Wallach, D. F. H. & Kamat, V. B. (1966) *Methods Enzymol.* **7**, 165–166