A basolateral lactate/H⁺ co-transporter in Madin–Darby Canine Kidney (MDCK) cells

Stuart O. ROSENBERG, Tina FADIL and Victor L. SCHUSTER*

Departments of Medicine and Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, NY 10461, U.S.A.

Monolayers of Madin–Darby Canine Kidney (MDCK) cells grown on permeable filters generated lactate aerobically and accumulated it preferentially in the basolateral compartment, suggesting the presence of a lactate carrier. The mechanism of lactate transport across apical and basolateral membranes was examined by determining intracellular pH (pH_i) microspectrofluorimetrically after addition of lactate to the extracellular solutions and by measuring uptake of [¹⁴C]lactate. Addition of 20 mM lactate to the apical compartment produced no change in pH_i, whereas lactate added to the basolateral compartment rapidly and reversibly lowered pH_i. Pyruvate produced similar results. Inhibitors of lactate/H⁺ co-transporters, α -cyano-4-hydroxycinnamate (CnCN) and quercetin, partially inhibited the fall in pH_i produced by basolateral lactate. In contrast, the disulphonic stilbene, DIDS (4,4'-di-isothiocyanostilbene-2,2'-

disulphonic acid) produced no inhibition at 0.5 mM. Kinetic analysis was performed by applying basolateral lactate at various concentrations and measuring the rate of entry (Δ pH_i/min) in the presence and absence of CnCN. Lactate flux was shown to occur by both non-ionic diffusion and a α -cyano-4-hydroxycinnamate-sensitive component (carrier). The latter has a K_m of ~ 7 mM for the lactate anion. Propionate, but not formate, lowered pH_i to the same degree as did equimolar lactate, but the propionate effect was not inhibited by CnCN. Influx of [¹⁴C]lactate was substantially greater across the basolateral membrane than across the apical membrane and occurred in the absence of Na⁺. We conclude that MDCK cells grown on permeable filters generate lactate aerobically and transport it across the basolateral membrane by way of a lactate/H⁺ co-transporter.

INTRODUCTION

Carrier-mediated monocarboxylate transport has been described in many membrane systems, including those of bacteria (ten Brink et al., 1985), mitochondria (Halestrap and Denton, 1974; Bolli et al., 1989) and eukaryotic cells. Since the initial report of lactate/H⁺ co-transport by Halestrap and Denton (1974), plasma-membrane monocarboxylate/H⁺ co-transporters have been described in erythrocytes (Deuticke, 1982; Jennings and Adams-Lackey, 1982), heart (Poole et al., 1989, 1990), liver (Quintana et al., 1988; Edlund and Halestrap, 1988; Metcalfe et al., 1986), skeletal muscle (Hirche et al., 1975; Mainwood and Worsley-Brown, 1975; Mason and Thomas, 1988; Roth and Brooks, 1990b), cornea (Chen and Chen, 1990), placenta (Balkovetz et al., 1988) and intestine (Tiruppathi et al., 1988).

There is some information about the polarity and functional characteristics of monocarboxylate transporters in kidney epithelia. The proximal-tubule brush-border membrane has both Na⁺/lactate and lactate/H⁺ co-transporters (Murer et al., 1981; Ullrich et al., 1982; Jorgensen and Sheikh, 1984; Siebens and Boron, 1987; Aronson, 1989), and the basolateral membrane has a lactate/H⁺ co-transporter (Murer et al., 1981; Siebens and Boron, 1987). The medullary thick ascending limb has a basolateral-membrane lactate/H⁺ co-transporter (Vinay et al., 1990).

The LLC-PK1 cell line, which has properties of the proximal tubule, has been used as an intact-cell model system for the study of the Na⁺/lactate co-transporter (Poustis-Delpont et al., 1988). There is, however, no good system *in vitro* for the study of

lactate/H⁺ co-transporters in transporting epithelia. In the present paper, we have examined lactate transport in the Madin–Darby Canine Kidney (MDCK) cell line, arguably the best characterized of all polarized transporting epithelia. MDCK cells have a glycolytic profile similar to that of the collecting duct (Lynch and Balaban, 1987a; Gstraunthaler et al., 1985). Because ion transport and the Na⁺/K⁺-ATPase are tightly coupled to glycolysis in MDCK cells (Lynch and Balaban, 1987a,b; Kurtz et al., 1987), this epithelium appears to be particularly appropriate for investigating the coupling between metabolism, lactate transport and ion fluxes. Here we show that MDCK cells have a basolateral lactate/H⁺ co-transporter, and compare its properties with those described previously in the plasma membranes of other mammalian cell types.

MATERIALS AND METHODS

Cell culture and lactate production

MDCK cells (type II, passages 11–14, CCL 34) obtained from the American Type Culture Collection (Rockville, MD, U.S.A.) were cultured on plastic in Dulbecco's modified Eagle's medium (DMEM) with 10% (v/v) fetal-calf serum and 1% penicillin/streptomycin. At confluence, cells were harvested by using EDTA and trypsin and seeded at 5×10^5 cells/cm² on to either uncoated 12 mm-diameter Anocel filters (Wheaton, Millville, NJ, U.S.A.) or Millipore-CM filters coated with collagen as previously described (Rosenberg et al., 1991). The filter unit with cells was placed into one well of a six-well culture dish containing DMEM. Lactate appearance in the medium was measured as the NADH fluorescence at 340 nm arising from the

Abbreviations used: MDCK, Madin–Darby Canine Kidney; DMEM, Dulbecco's Modified Eagle's Medium; BCECF-AM, 2',7'-bis(2-carboxyethyl)-5,6-carboxyfluorescein acetoxymethyl ester; DIDS, 4,4'-di-isothiocyanostilbene-2,2'-disulphonic acid; CnCN, α -cyano-4-hydroxycinnamate; pH_i, intracellular pH.

* To whom correspondence should be addressed.

conversion of lactate into pyruvate by using an enzyme-linked assay kit (Sigma, St. Louis, MO, U.S.A.).

Epifluorescence and microspectrofluorimetry

Confluent monolayers (3–4 days as assessed by visual inspection and by transepithelial resistance; Rosenberg et al., 1991) were loaded at 37 °C for 40 min from the apical side with 20–30 μ M of the pH-sensitive fluorescent dye 2',7'-bis(2-carboxyethyl)-5,6-carboxyfluorescein acetoxymethyl ester (BCECF-AM). BCECF-AM was obtained from Molecular Probes (Eugene, OR, U.S.A.) and made up as a 30 mM stock in dimethyl sulphoxide on the day of use. As previously described (Rosenberg et al., 1991), infusion and suction lines were positioned in the apical and basolateral compartments, and solutions were delivered by gravity-feed through tubing from closed reservoirs to manual switching valves and then to the respective open-air chambers. All experiments were performed at 25 °C. The inverted filter system previously described (Rosenberg et al., 1991) was not used for these experiments. Cells were instead observed through the translucent Anocel filters, which allowed adequate resolution of individual cells without high background fluorescence.

Digital imaging microspectrofluorimetry was performed as previously described (Rosenberg et al., 1991). All images were acquired with background fluorescence subtracted electronically. During measurement of intracellular pH (pH_i), the monolayers were perfused on each side with a CO_2/HCO_3^- -free saline solution, buffered with Hepes equilibrated with air (132 mM Na^+ , 127 mM Cl^- , 5 mM K^+ , 20 mM Hepes, 1 mM PO_4^{3-} , 1 mM Ca^{2+} and 20 mM glucose). When monocarboxylic acids were added (Na^+ or Li^+ salts of lactate, propionate or formate), they were substituted for an equimolar amount of NaCl. Quercetin and α -cyano-4-hydroxycinnamate (CnCN) were added as the powdered reagent to perfusing solutions on the day of the experiment. At the end of an experiment, pH_i was calibrated as described previously (Boyarsky et al., 1988; Rosenberg et al., 1991) by using 10 μ M nigericin in a Ringer solution containing 110 mM K^+ in which the amounts of Na^+ -Hepes and H^+ -Hepes were adjusted to give a pH range of 6.5–8.0.

[^{14}C]Lactate uptake

[^{14}C]Lactate and [3H]methoxyinulin were obtained from Amersham (Arlington Heights, IL, U.S.A.). [3H]Inulin, used as a marker of extracellular space, was dialysed before use, with a molecular cut-off of 3500 Da. Cells seeded on to filters 2–3 days before the experiment were washed briefly with Ringer solution buffered with Hepes and placed into 24-well plates. The appropriate medium containing [^{14}C]lactate (4 μ Ci/ml) and [3H]inulin (1 μ Ci/ml) was placed on either the basolateral or the apical side. Identical unlabelled medium was placed on the opposite side. No unlabelled lactate was added with the tracer. The cells were left for 30 minutes and were then washed with ice-cold unlabelled medium. Mean extracellular lactate concentrations at 30 min resulting from glycolysis and tracer addition were $\leq 70 \mu$ M in the apical and $\leq 180 \mu$ M in the basolateral compartments.

The filter was placed in 0.5 ml of 0.1 M HNO_3 overnight. On the following day, 10 ml of scintillation fluid was added to each vial and the samples were counted for radioactivity for 10 min. 3H and ^{14}C blanks were subtracted, and 3H counts were corrected for spill-over from the ^{14}C channel. The fraction of filter-associated [^{14}C]lactate counts due to extracellular trapping was determined from the [3H]inulin counts. In the absence of excess unlabelled lactate, ^{14}C tracer uptake at 23 °C was linear over 30 min ($r = 0.98$).

In separate experiments, the filter was removed, placed in HNO_3 overnight, and the protein content per filter was determined by using a commercial kit (Bio-Rad, Richmond, CA, U.S.A.). Protein content averaged 20 μ g/0.6 cm 2 filter.

Solutions and reagents

DMEM, antibiotics, fetal-bovine serum, lactate, propionate, formate, CnCN, quercetin, nigericin, 4,4'-di-isothiocyanostilbene-2,2'-disulphonic acid (DIDS) and salts were purchased from Sigma.

Statistics

Values of pH_i for 1–5 areas on a filter, each of which contained 1–5 cells, were averaged to yield a single mean value for that monolayer. Sequential data on one or more cells of a single monolayer were compared by paired t test. Data obtained from separate monolayers were compared by non-paired t test or analysis of variance (ANOVA). Bars on Figures represent standard errors. Significance level was 0.05.

RESULTS

Sided lactate accumulation

We first examined the sidedness of lactate accumulation by MDCK monolayers grown in standard DMEM. The media of the apical and basolateral compartments were changed, and lactate concentrations on each side were determined after various intervals. Volumes of the apical and basolateral compartments were equal. After 30 min the lactate concentrations in the fresh media had increased to approx. 40–150 μ M and the paired basolateral/apical lactate concentration ratio was 2.3 ± 0.62 ($n = 4$, $P < 0.05$ compared with zero). After 16–24 h in fresh media the paired ratio was 1.92 ± 0.26 ($n = 9$, $P < 0.001$ compared with zero). These results are similar to those previously reported (Kurtz et al., 1987). In the remaining experiments, we tested the hypothesis that sided lactate accumulation could be due to a basolateral lactate carrier.

Effects of extracellular lactate on pH_i

MDCK monolayers were loaded with the fluorescent dye BCECF. Changes in pH_i were determined by using ratio fluorescence digital imaging spectroscopy (Rosenberg et al., 1991) after changing the solution perfusing either the apical or basolateral side of the monolayer to contain 20 mM L-lactate. Influx of the lactate anion into cells should increase pH_i as intracellular protons are buffered. In contrast, lactate influx as protonated lactic acid or via lactate- H^+ co-transport should decrease pH_i . Figure 1 shows the results of a representative experiment. As seen in the first part of the record, addition of 20 mM L-lactate at pH 7.4 to the basolateral compartment produced a rapid decrease in pH_i . Upon removal of lactate, there was a prompt recovery of pH_i with an overshoot (the overshoot is presumably due to delayed inactivation of H^+ -extruding mechanisms). In contrast with the results seen with basolateral addition, addition of 20 mM L-lactate to the apical chamber produced no change in pH_i . In paired experiments on seven monolayers, the rates of change in pH_i were significantly different upon adding apical or basolateral lactate (apical = -0.008 ± 0.006 pH unit/min, basolateral = -0.269 ± 0.033 pH unit/min; $P < 0.001$). These results indicate that lactate enters MDCK cells predominantly in the protonated form, and that entry is sided, i.e. basolateral $>$ apical.

Figure 1 Sidedness and inhibition of lactate entry by pH_i determination

Addition of 20 mM L-lactate to the basolateral, but not apical, compartment produced a rapid decrease in pH_i . Addition of 20 mM lactate in the presence of 5 mM CnCN produced a blunted effect on pH_i .

Kinetics of lactate entry into cells

Lactate could enter cells in the protonated form either by non-ionic diffusion or by a carrier-mediated process. We used CnCN, known to block lactate/ H^+ co-transporters in other model systems (Halestrap and Denton, 1974; Jennings and Adams-Lackey, 1982; Siebens and Boron, 1987; Mason and Thomas, 1988; Metcalfe et al., 1988; Edlund and Halestrap, 1988; Poole and Halestrap, 1988; Poole et al., 1989, 1990), to estimate that proportion of lactate entry due to a carrier-mediated process. The middle portion of Figure 1 shows the results of repeating the basolateral lactate pulse in the presence of 5 mM CnCN. Addition of CnCN alone had no effect on pH_i (mean paired difference = 0.053 ± 0.049 pH unit; $n = 7$, $P = \text{NS}$). Addition of 20 mM L-lactate in the presence of CnCN produced less of an effect on pH_i than in the absence of CnCN (Figure 1). The inhibition of $\Delta\text{pH}_i/\text{min}$ by CnCN was $\sim 50\%$ (-0.136 ± 0.035 versus -0.269 ± 0.033 pH unit/min; $n = 7$, $P < 0.001$) and was completely reversible (Figure 1). Quercetin, another inhibitor of lactate transport (Belt et al., 1979; Jennings and Adams-Lackey, 1982; Edlund and Halestrap, 1988), also inhibited by $\sim 50\%$ (S. O. Rosenberg and V. L. Schuster, unpublished work) the $\Delta\text{pH}_i/\text{min}$ produced by 20 mM basolateral lactate (quercetin at 5 mM; $n = 2$).

We repeated protocols similar to that shown in Figure 1 at four different concentrations of basolateral lactate on each monolayer. In each case, the extracellular pH was 7.4. Figure 2 shows the changes in pH_i/min as a function of the applied basolateral lactate anion concentration. The top curve, depicting the $\Delta\text{pH}_i/\text{min}$ in the absence of CnCN, shows no saturation at the highest lactate concentrations.

The CnCN-sensitive component (bottom curve) was calculated as: (control $\Delta\text{pH}_i/\text{min}$) - ($\Delta\text{pH}_i/\text{min}$ in the presence of 5 mM CnCN). Analysis of kinetic parameters by the direct linear plot method (Eisenthal and Cornish-Bowden, 1974) yielded a V_{max} of 0.202 pH unit/min and a K_m of 6.95 mM for the lactate anion. Given the pK_a of lactate (3.86), this value corresponds to a K_m of $\sim 1.9 \mu\text{M}$ lactic acid at pH 7.4. The kinetic curve predicted from these values is drawn over the data for the CnCN-sensitive component in Figure 2.

In contrast, the calculated CnCN-insensitive component is best fitted by the linear equation $\Delta\text{pH}_i/\text{min} = 0.01[\text{lactate}$

Figure 2 Kinetic analysis of basolateral lactate entry

The initial rate of change of pH_i is depicted as a function of basolateral lactate concentration.

Figure 3 Comparison of basolateral pyruvate and lactate permeabilities

Addition of 16 mM L-lactate to the basolateral compartment produced a rapid intracellular acidification indistinguishable from that seen with the subsequent addition of 16 mM pyruvate.

(mM)] + 0.075 ($r = 0.975$, $P < 0.001$). The CnCN-insensitive line fails to go through zero; this is consistent with incomplete blockage of the carrier by CnCN at low [lactate], and thus overestimation of the diffusional component. Nonetheless, the linear dependence of flux on substrate concentration is characteristic of diffusion.

In separate experiments, we also determined the total [^{14}C]lactate uptake by MDCK cells on filters. In the presence of 16 mM unlabelled lactate, the rate of [^{14}C]lactate uptake over 30 min across the basolateral membrane was non-linear and fitted a single exponential with an initial rate of 132 nmol/min per mg of protein (results not shown).

Monocarboxylate specificity

To assess carrier specificity, we examined the effects of three other monocarboxylic acids on pH_i . In the representative experiment shown in Figure 3, the $\Delta\text{pH}_i/\text{min}$ induced by 16 mM basolateral pyruvate was indistinguishable from that produced by equimolar lactate. In five monolayers, the $\Delta\text{pH}_i/\text{min}$ and the degree of inhibition by CnCN were not significantly different between pyruvate and lactate (Table 1). This is in agreement with reports in other systems in which pyruvate is a good substrate for the lactate/ H^+ co-transporter (Edlund and Halestrap, 1988; Balkovetz et al., 1988; Poole et al., 1989).

We also examined the relative permeabilities of formate and

Table 1 Lactate and pyruvate effects on pH_i

$n = 5$ for each group. Anion concentrations were 16 mM; [CnCN] = 5 mM. Paired analyses: * $P < 0.05$ compared with basolateral anion without CnCN.

Basolateral anion	$\Delta pH_i/\text{min}$	
	Control	+ CnCN
Lactate	-0.436 ± 0.057	$-0.100 \pm 0.006^*$
Pyruvate	-0.487 ± 0.031	$-0.169 \pm 0.017^*$

propionate. Formate has a pK_a that is similar to that of lactate (3.75 and 3.86 respectively), but an oil/water partition coefficient twice that of lactate (8×10^{-3} versus 4×10^{-3}) (Matsuzaki et al., 1988). Thus, at the same external pH_i , formic acid should enter the cell by non-ionic diffusion at roughly twice the rate of lactic acid. Figure 4 depicts a representative experiment comparing the effects of 20 mM formate and lactate on pH_i . It is clear that the apparent entry rate of formate as estimated by $\Delta pH_i/\text{min}$ is less than that of lactate. The mean formate data for several monolayers are shown in Table 2.

The pK_a of propionate (4.87) is 10 times, and the oil/water partition coefficient 40 times, that of lactate (Matsuzaki et al., 1988). The rate of entry of propionic acid into MDCK cells by non-ionic diffusion would therefore be predicted to be substantially higher than that of lactic acid. As shown in Table 2, the overall rate of propionate entry into MDCK cells was comparable with that of lactate. Basolateral entry of lactate was inhibited by CnCN, but basolateral entry of formate or propionate was not (Table 2). These results are consistent with the presence of a carrier-mediated process for lactate entry across the basolateral membrane.

Table 2 also shows that the entry of propionate by non-ionic diffusion (CnCN-insensitive component) exhibits basolateral > apical sidedness. This result is probably due to non-ionic diffusion occurring through a basolateral surface area that is larger than the apical surface area (von Bonsdorff et al., 1985).

Effect of disulphonic stilbenes

Because lactate can be transported via the erythrocyte band-3 $\text{Cl}^-/\text{HCO}_3^-$ exchanger, we examined the effect of DIDS on MDCK-cell lactate transport. Confluent monolayers were first exposed to 16 mM basolateral L-lactate and changes in pH_i were recorded. Then 0.5 mM DIDS was applied for 10–15 min, after which pH_i measurements were again made during basolateral L-lactate exposure. The $\Delta pH_i/\text{min}$ induced by lactate was not significantly inhibited by DIDS (control = -0.384 ± 0.081 pH unit/min; DIDS = -0.343 ± 0.070 pH unit/min; $n = 5$, $P = \text{not significant}$).

Na^+ -independence: [^{14}C]lactate uptake

Although the data presented above suggest that most of the lactate entry into MDCK cells occurs by lactate/ H^+ co-transport, the data do not exclude a small component of simultaneous Na^+ /lactate co-transport. Attempts to determine lactate-induced $\Delta pH_i/\text{min}$ in a Na^+ -free buffer were unsuccessful because of a marked lowering of baseline pH_i , probably owing to reversal of Na^+/H^+ exchange (Rosenberg et al., 1991). Therefore we examined Na^+ -dependence, using sided [^{14}C]lactate uptake by MDCK cells on filters. The filter-associated ^{14}C tracer uptake, determined at 30 min in the absence of excess unlabelled lactate,

Figure 4 Comparison of basolateral formate and lactate permeabilities

Addition of 20 mM formate to the basolateral compartment produced a minimal change in pH_i compared with that seen with the subsequent addition of 20 mM lactate.

Table 2 $\Delta pH_i/\text{min}$ produced by lactate, propionate and formate

$n = 4$ monolayers for apical measurements and 6–7 for basolateral measurements. Anion concentrations were 20 mM; [CnCN] = 5 mM. Paired analyses: * $P < 0.05$ compared with addition of anion to the apical side; † $P < 0.05$ compared with addition of anion to the basolateral side, without CnCN.

Anion	$\Delta pH_i/\text{min}$		
	Apical	Basolateral	Basolateral + CnCN
Lactate	-0.008 ± 0.006	$-0.269 \pm 0.033^*$	$-0.136 \pm 0.035^\dagger$
Propionate	-0.050 ± 0.060	$-0.237 \pm 0.064^*$	-0.193 ± 0.068
Formate	-0.008 ± 0.009	$-0.066 \pm 0.013^*$	-0.046 ± 0.008

Table 3 Uptake of [^{14}C]lactate by filter-grown MDCK cells

Side of tracer	Buffer	n	30 min uptake (pmol/min per mg of protein)
Apical	Na^+ Hepes	5	$4.72 \pm 0.6^\dagger$
Basolateral	Na^+ Hepes	13	27.8 ± 2.8
Basolateral	K^+ nigericin	11	21.4 ± 2.9

† $P < 0.05$ compared with the other two groups (ANOVA).

is expressed as pmol/min per mg of protein in Table 3. The uptake of [^{14}C]lactate by MDCK cells on filters from the basolateral side was about 6 times that from the apical side (27.8 versus 4.7 pmol/min per mg). Also shown in Table 3 are the results of experiments in which we removed extracellular Na^+ and clamped intracellular pH_i constant at 7.4 with K^+ Hepes Ringer and nigericin (Thomas et al., 1979). The uptake of [^{14}C]lactate from the basolateral side was unaffected by the removal of Na^+ . Thus lactate influx across the basolateral membrane is Na^+ -independent.

DISCUSSION

The central finding is that the renal-collecting-duct cell line MDCK has a lactate/ H^+ co-transporter that is polarized to the basolateral membrane. The evidence can be summarized as follows. MDCK cells grown on permeable filters accumulate

lactate preferentially in the basolateral compartment. Lactate permeability of the basolateral membrane is substantially greater than that of the apical membrane. Lactate flux across the basolateral membrane is independent of Na^+ and is associated with changes in pH_i that are consistent with lactate/ H^+ co-transport (lactate/ OH^- exchange). By kinetic analysis, basolateral lactate flux occurs both by non-ionic diffusion and by a carrier. The carrier has a K_m of ~ 7 mM for the lactate anion, and is inhibited by CnCN and quercetin, but not by DIDS. Pyruvate is a good substrate for the carrier, but formate and propionate are not.

Several experimental considerations warrant further discussion. First, in the kinetic experiments (Figure 2), ΔpH_i was determined after adding external lactate at various concentrations. The underlying assumption of this analysis is that the intracellular concentration of lactate is essentially zero upon addition of external lactate. Several observations suggest that the intracellular lactate concentration in MDCK cells perfused in Hepes Ringer containing only glucose as a carbon source is quite low. First, MDCK monolayers incubated in fresh media were able to increase external lactate concentration to only 40–150 μM over 30 min. Given that the pH_i experiments were performed in a continuously perfused system, any lactate produced would be expected to diffuse out into an infinite sink. Second, addition of α -CnCN to MDCK cells in the presence of glucose failed to lower pH_i acutely, results that are not consistent with high background rates of lactic acid production. Third, unlike Ehrlich ascites-tumour cells, which generate sufficient lactic acid to cause a transient fall in pH_i when pulsed with glucose in the presence of rotenone, an inhibitor of oxidative phosphorylation (Thomas et al., 1979), MDCK cells exhibit no such change (S. O. Rosenberg and V. L. Schuster, unpublished work). Therefore it seems reasonable to assume that intracellular lactate concentrations were low on addition of external lactate.

Second, in our kinetic analysis we operationally defined carrier-mediated lactate transport as that which is inhibited by CnCN. To the extent that CnCN may not be a 100% effective blocker of the MDCK lactate/ H^+ co-transporter, we may have underestimated the maximal rate (V_{max}) of the MDCK monocarboxylate carrier. In fact, the linear equation describing the CnCN-insensitive component suggests that this may be the case. Nonetheless, our V_{max} is a reasonable approximation.

A third point concerns the amount of sided lactate output that may occur by non-ionic diffusion. The basolateral-membrane area of MDCK cells is amplified approx. 4-fold over that of the apical membrane (von Bonsdorff et al., 1985). Moreover, the basolateral membrane of MDCK cells is poor in glycosphingolipids, has a higher phosphatidylcholine content, and has an increased membrane fluidity relative to the apical membrane (Mays and Nelson, 1991). This means that some difference in the sidedness of the apparent lactate permeability would be expected simply on the basis of differences in non-ionic diffusion. Indeed, we found basolateral permeability greater than apical permeability for all monocarboxylates examined. The formate and propionate fluxes, however, were not inhibited by CnCN, and the formate permeability was less than that of lactate, despite substantially more favourable physical properties for entry by non-ionic diffusion ($\text{p}K_a$ and oil/water partition coefficient). The difference between the basolateral and apical membrane areas and lipid contents can therefore only account for some of the sided lactate permeability. The remainder must be due to carrier-mediated lactate flux across the basolateral membrane.

A fourth point concerns the magnitude of the lactate fluxes determined by the pH_i method as compared with ^{14}C -tracer

uptakes. We can convert $\Delta\text{pH}_i/\text{min}$ into nmol/min per mg of protein from the pH -determined V_{max} (0.202 pH unit/min), the buffer capacity of 10 mM/ pH unit (Rosenberg et al., 1991), the cell volume for MDCK type II cells of 1169 $\mu\text{m}^3/\text{cell}$ (von Bonsdorff et al., 1985), a cell density of 1.2×10^6 cells/ cm^2 , and a protein concentration of 20 $\mu\text{g}/0.6 \text{ cm}^2$ filter. The carrier V_{max} is thus 80 nmol/min per mg of protein. In turn, from the K_m , the V_{max} , and the diffusion equation as discussed above, at a basolateral lactate concentration of 16 mM the predicted influx rate is 149 nmol/min per mg of protein (diffusion = 93 nmol/min per mg, carrier = 56 nmol/min per mg). Similarly, by ^{14}C -tracer uptake the initial rate for carrier plus diffusional entry at 16 mM lactate was 132 nmol/min per mg of protein. Thus lactate influx rates determined by $\Delta\text{pH}/\text{min}$ and by initial tracer uptakes give comparable results.

Our estimated V_{max} lactate influx rate of 80 nmol/min per mg is higher than that reported in rat erythrocytes at 7 °C (~ 5 nmol/min per mg; Edlund and Halestrap, 1988), rat hepatocytes at 0 °C (~ 3 nmol/min per mg; Edlund and Halestrap, 1988) or guinea-pig cardiac myocytes at 11 °C (7.5 nmol/min per mg; Poole et al., 1989). However, taking into account the temperature-dependence of the lactate/ H^+ co-transporter as reported in the erythrocyte (Halestrap, 1976) and rat sarcolemmal vesicles (Roth and Brooks, 1990a), differences in assay temperature can probably explain our higher V_{max} . Indeed, our estimated V_{max} is in reasonable agreement with that measured at 25 °C in rat sarcolemmal vesicles (139 nmol/min per mg of protein; Roth and Brooks, 1990a) and human placental vesicles (108 nmol/min per mg of protein; Balkovetz et al., 1988).

The MDCK monocarboxylate transporter shares many properties with lactate/ H^+ co-transporters seen in other cell types. As has been reported for other lactate/ H^+ co-transporters (Tiruppathi et al., 1988; Mason and Thomas, 1988; Edlund and Halestrap, 1988; Balkovetz et al., 1988; Roth and Brooks, 1990a), we found poor inhibition by the disulphonic stilbene DIDS. As in erythrocytes and Ehrlich ascites cells (Jennings and Adams-Lackey, 1982; Belt et al., 1979), the MDCK transporter is inhibited by both CnCN and quercetin. In the MDCK system, pyruvate is transported as well as lactate, a finding that has also been reported for other monocarboxylate transporters (Balkovetz et al., 1988; Edlund and Halestrap, 1988; Poole et al., 1989).

The lactate K_m reported for lactate/ H^+ co-transporters varies substantially, depending on the cell type. In rat hepatocytes and erythrocytes (Edlund and Halestrap, 1988), rat liver vesicles (Quintana et al., 1988) and guinea-pig cardiac myocytes (Poole et al., 1989) the K_m is ~ 2 –3 mM. In human erythrocytes (Halestrap, 1976) and frog sartorius muscle (Mason and Thomas, 1988) K_m values of ~ 9 –10 mM have been reported, a range similar to our present value of 6.95 mM in MDCK cells. In contrast, substantially higher K_m values (24–40 mM) have been reported in rat sarcolemmal vesicles (Roth and Brooks, 1990a; Juel, 1991). This variability could be due to methodological differences, or could reflect the existence of multiple lactate/ H^+ co-transporter isoforms.

The present results explain the data of Kurtz et al. (1987) of preferential basolateral lactate output by MDCK cells. The MDCK cell is derived from the collecting duct (Valentich, 1981; Ojakian et al., 1987) and is glycolytic (Gstraunthaler et al., 1985; Lynch and Balaban, 1987a). Because transformed cell lines from the mouse and rabbit cortical collecting ducts are also glycolytic and display preferential basolateral lactate output (Bello-Reuss and Cabell-Kluch, 1990; Stoos et al., 1991), it appears likely that basolateral lactate/ H^+ co-transporters are a general feature of collecting duct cell lines.

Transformed cell lines are known to be highly glycolytic (Paul, 1965; Belt et al., 1979; Gstraunthaler et al., 1985). Moreover, epithelia such as the proximal tubule, which are gluconeogenic *in situ*, rapidly become glycolytic when grown in tissue culture (Tang et al., 1989). Do our results on MDCK cells apply to the collecting duct *in situ*? Several lines of evidence indicate that, like collecting-duct cell lines, the collecting duct *in situ* is also glycolytic. (1) Single dissected rat and mouse collecting ducts show the highest rates of aerobic glycolysis of all nephron segments (Bagnasco et al., 1985; Uchida and Endou, 1988). (2) Histochemical and immunocytochemical data show that glycolytic enzymes (hexokinase, phosphofructokinase, pyruvate kinase) are located preferentially in the collecting duct (Schmid et al., 1980; Lawrence and Trayer, 1984; Gstraunthaler et al., 1985; Wirthensohn and Guder, 1986; Lawrence et al., 1986). (3) Lactate is generated aerobically at substantial rates by freshly isolated inner-medullary collecting-duct cells (Grupp et al., 1989). (4) Primary cultures of rabbit collecting ducts grown on permeable filters exhibit sided (basolateral) lactate appearance similar to that seen in the present study with MDCK cells (Fejes-Tóth and Náray-Fejes-Tóth, 1987).

Taken together, these data suggest that the collecting duct *in situ* has a basolateral lactate/H⁺ co-transporter. Such a transporter would serve to salvage lactic acid, generated by aerobic glycolysis, and transport it back into the plasma for participation in the Cori cycle. Studies on isolated perfused kidneys indicate that an intrarenal Cori cycle can exist between 'distal' glycolytic segments like the collecting duct and the gluconeogenic proximal tubule (Bartlett et al., 1984). Further study of perfused collecting ducts and collecting ducts in primary culture will be required in order to address the question of collecting-duct lactate transporters more directly.

Grant support was from N.I.H. grant DK 38095 and a Grant-In-Aid from the New York Heart Association. S.O.R. was supported by a Research Fellowship of the National Kidney Foundation, New York/New Jersey Affiliate. V.L.S. is an Established Investigator of the American Heart Association and a Scholar of the Irma T. Hirschl-Monique Weill-Caulier Trust.

REFERENCES

- Aronson, P. S. (1989) *Annu. Rev. Physiol.* **51**, 419–441
- Bagnasco, S., Good, D., Balaban, R. and Burg, M. (1985) *Am. J. Physiol.* **248**, F522–F526
- Balkovetz, D. F., Leibach, F. H., Mahesh, V. B. and Ganapathy, V. (1988) *J. Biol. Chem.* **263**, 13823–13830
- Bartlett, S., Espinal, J., Janssens, P. and Ross, B. D. (1984) *Biochem. J.* **219**, 73–78
- Bello-Reuss, E. and Cabell-Kluch, L. (1990) *Kidney Int.* **37**, 533 (abstr.)
- Belt, J. A., Thomas, J. A., Buchsbaum, R. N. and Racker, E. (1979) *Biochemistry* **18**, 3506–3511
- Bolli, R., Nalecz, K. A. and Azzi, A. (1989) *J. Biol. Chem.* **264**, 18024–18030
- Boyarsky, G., Ganz, M. B., Sterzel, R. B. and Boron, W. F. (1988) *Am. J. Physiol.* **255**, C844–C856
- Chen, C. H. and Chen, S. C. (1990) *Arch. Biochem. Biophys.* **276**, 70–76
- Deuticke, B. (1982) *J. Membr. Biol.* **70**, 89–103
- Edlund, G. L. and Halestrap, A. P. (1988) *Biochem. J.* **249**, 117–126
- Eisenthal, R. and Cornish-Bowden, A. (1974) *Biochem. J.* **139**, 715–720
- Fejes-Tóth, G. and Náray-Fejes-Tóth, A. (1987) *Am. J. Physiol.* **253**, F1302–F1307
- Grupp, C., Pavenstadt-Grupp, I., Grunewald, R. W., Bevan, C., Stokes, J. B. and Kinne, R. K. H. (1989) *Kidney Int.* **36**, 201–209
- Gstraunthaler, G., Pfaller, W. and Kotano, P. (1985) *Am. J. Physiol.* **248**, F536–F544
- Halestrap, A. P. (1976) *Biochem. J.* **156**, 193–207
- Halestrap, A. P. and Denton, R. M. (1974) *Biochem. J.* **138**, 313–316
- Hirche, H. J., Hombach, V., Langohr, H. D., Wacker, U. and Busse, J. (1975) *Pflügers Arch.* **356**, 209–222
- Jennings, M. L. and Adams-Lackey, M. (1982) *J. Biol. Chem.* **257**, 12866–12871
- Jorgensen, K. E. and Sheikh, M. I. (1984) *Biochem. J.* **223**, 803–807
- Juel, C. (1991) *Biochim. Biophys. Acta* **1065**, 15–20
- Kurtz, A., Pfeilschifter, J., Malmstrom, K., Woodson, R. D. and Bauer, C. (1987) *Am. J. Physiol.* **252**, C307–C314
- Lawrence, G. M. and Trayer, I. P. (1984) *Histochem. J.* **16**, 697–708
- Lawrence, G. M., Jepson, M. A., Trayer, I. P. and Walker, D. G. (1986) *Histochem. J.* **18**, 43–53
- Lynch, R. M. and Balaban, R. S. (1987a) *Am. J. Physiol.* **252**, C225–C231
- Lynch, R. M. and Balaban, R. S. (1987b) *Am. J. Physiol.* **253**, C269–C276
- Mainwood, G. W. and Worsley-Brown, P. (1975) *J. Physiol. (London)* **250**, 1–22
- Mason, M. J. and Thomas, R. C. (1988) *J. Physiol. (London)* **400**, 459–479
- Matsuzaki, K., Stokes, J. B. and Schuster, V. L. (1988) *J. Clin. Invest.* **82**, 57–64
- Mays, R. W. and Nelson, W. J. (1991) *J. Cell Biol.* **115**, 65a
- Metcalfe, H. K., Monson, J. P., Welch, S. G. and Cohen, R. D. (1986) *J. Clin. Invest.* **78**, 743–747
- Metcalfe, H. K., Monson, J. P., Cohen, R. D. and Padgham, C. (1988) *J. Biol. Chem.* **263**, 19505–19509
- Murer, H., Barac-Nieto, M., Ullrich, K. J. and Kinne, R. (1981) in *Renal Transport of Organic Substances* (Greger, R., Lang, F. and Silbernagl, S., eds.), pp. 211–223, Springer-Verlag, New York
- Ojakian, G. K., Romain, R. E. and Herz, R. E. (1987) *Am. J. Physiol.* **253**, C433–C443
- Paul, J. (1965) in *Cells and Tissues in Culture* (Willmer, E. N., ed.), pp. 239–276, Academic Press, New York
- Poole, R. C. and Halestrap, A. P. (1988) *Biochem. J.* **254**, 385–390
- Poole, R. C., Halestrap, A. P., Price, S. J. and Levi, A. J. (1989) *Biochem. J.* **264**, 409–418
- Poole, R. C., Cranmer, S. L., Halestrap, A. P. and Levi, A. J. (1990) *Biochem. J.* **269**, 827–829
- Poustis-Delpont, C., Mengual, R. and Sudaka, P. (1988) *Am. J. Physiol.* **255**, F1249–F1255
- Quintana, I., Felipe, A., Remesar, X. and Pastor-Anglada, M. (1988) *FEBS Lett.* **235**, 224–228
- Rosenberg, S. O., Berkowitz, P. A., Li, L. and Schuster, V. L. (1991) *Am. J. Physiol.* **260**, C868–C876
- Roth, D. A. and Brooks, G. A. (1990a) *Arch. Biochem. Biophys.* **279**, 377–385
- Roth, D. A. and Brooks, G. A. (1990b) *Arch. Biochem. Biophys.* **279**, 386–394
- Schmid, H., Mall, A., Scholz, M. and Schmidt, U. (1980) *Hoppe-Seyler's Z. Physiol. Chem.* **361**, 819–827
- Siebens, A. W. and Boron, W. F. (1987) *J. Gen. Physiol.* **90**, 799–831
- Stoos, B. A., Náray-Fejes-Tóth, A., Carretero, O. A., Ito, S. and Fejes-Tóth, G. (1991) *Kidney Int.* **39**, 1168–1175
- Tang, M.-J., Suresh, K. R. and Tannen, R. L. (1989) *Am. J. Physiol.* **256**, C532–C539
- ten Brink, B., Otto, R., Hansen, U.-P. and Konings, W. N. (1985) *J. Bacteriol.* **162**, 383–390
- Thomas, J. A., Buchsbaum, R. N., Zimniak, A. and Racker, E. (1979) *Biochemistry* **18**, 2210–2218
- Tiruppathi, C., Balkovetz, D. F., Ganapathy, V., Miyamoto, Y. and Leibach, F. H. (1988) *Biochem. J.* **256**, 219–223
- Uchida, S. and Endou, H. (1988) *Am. J. Physiol.* **255**, F977–F983
- Ullrich, K. J., Rumrich, G. and Klöss, S. (1982) *Pflügers Arch.* **395**, 212–219
- Valentic, J. D. (1981) *Ann. N.Y. Acad. Sci.* **372**, 384–404
- Vinay, P., Sénécal, J., Noël, J., Tejedor, A., Berteloot, A. and Gougoux, A. (1990) *Am. J. Physiol.* **258**, F1042–F1053
- von Bonsdorff, C.-H., Fuller, S. and Simons, K. (1985) *EMBO J.* **4**, 2781–2792
- Wirthensohn, G. and Guder, W. G. (1986) *Physiol. Rev.* **66**, 469–497