J. Membrane Biol. 59, 13–18 (1981)

The Journal of
Membrane Biology

Transepithelial Transport in Cell Culture: D-Glucose Transport by a Pig Kidney Cell Line ($LLC\text{-}PK_1$)

Dayton S. Misfeldt* and Martin J. Sanders

Department of Medicine (Oncology), Stanford University School of Medicine, Stanford, California 94305, and
Palo Alto Veterans Administration Medical Center, Palo Alto, California 94304

Summary. The pig kidney cell line $LLC\text{-}PK_1$ cultured on a collagen coated membrane filter formed a continuous sheet of oriented asymmetrical epithelial cells joined by occluding junctions. A transepithelial electrical potential (PD) and short-circuit current (SCC) were dependent on the presence of Na and sugar in the apical bathing solution. In the presence of 5.5 mM D-glucose, a PD of 2.8 mV, apical surface negative, a SCC of 13 μA cm^{-2} and transepithelial resistance of 211 ohm · cm^2 were recorded. The SCC was promptly reduced by the addition of phlorizin to the apical bath but unaffected when placed in the basolateral bath. The effect on SCC of various sugars was compared by the concentrations required for half-maximal SCC: 0.13 mM β-methyl-D-glucoside, 0.28 mM D-glucose, 0.65 mM α-methyl-D-glucoside, 0.77 mM 6-deoxy-D-glucose, 4.8 mM D-galactose, and 29 mM 3-O-methyl-glucose. When [Na] was reduced, the concentration of D-glucose required for half-maximal SCC increased. Isotopically labeled ^3H and ^{14}C D-glucose were used to simultaneously determine bidirectional fluxes; a resultant net apical-to-basolateral transport was present and abolished by phlorizin. The transported isotope cochromatographed with labeled D-glucose, indicating negligible metabolism of transported glucose. The pig kidney cell line, LLC-PK_1, provides a cell culture model for the investigation of mechanisms of transepithelial glucose transport.

Key words: Hexoses, short-circuit current, transepithelial transport, phlorizin, sodium dependent.

The advantages of the cell-culture system for studying epithelial transport depend on the question under investigation. In cell culture, reconstituted epithelial tissue potentially provides an experimental system of ultimate simplicity. Even epithelial cells from organs of complex architecture, i.e., kidney (Leighton et al., 1969; Hull, Cherry & Weaver, 1976), liver (Owens, Smith & Hacket, 1974) or mammary gland (Owens et al., 1974; Bisbee, Machen & Bern, 1979) are reduced to a single cell layer that forms a transporting epithelial tissue. Epithelial function studied by culturing cells on a supportive porous membrane allows access and separate bathing of either side of the cell layer (Misfeldt, Hamamoto & Pitelka, 1976; Cereijido et al., 1978). The heterogeneity of cell types can be reduced to the extent possible by cloning from cell lines and the culture conditions chemically defined (Taub et al., 1979) and thus controlled. Also, the continuous growth of cell lines in culture allows collection of whatever amount of tissue is necessary.

As a first step toward utilizing the advantages of cell culture for the understanding of epithelial transport function, we have identified in a pig kidney cell line, $LLC\text{-}PK_1$, net transepithelial D-glucose transport and have characterized the similarity of this system to kidney proximal tubule and intestinal epithelia. Independently, others have reported for LLC-PK_1 Na-dependent cellular concentration of α-methyl-D-glucoside which was inhibited by phlorizin (Mullin, Diamond & Kleinzeller, 1979; Rabito & Ausiello, 1980).

* To whom reprint requests should be directed.

Materials and Methods

Cell–Filter Preparation

Pig kidney cell line $LLC\text{-}PK_1$ was obtained from the American Type Tissue Collection (Rockville, Maryland) and grown on collagen-coated Millipore filters (0.45 μm, type HAMK, 25 mm). Filters were prepared by soaking in a 0.5% rat tail collagen solution (acetic acid/water, 1:1000) and exposed to ammonia vapor for

0022-2631/81/0059-0013 $01.20
© 1981 Springer-Verlag New York Inc.

30 min. This was followed by fixation in 3% glutaraldehyde for 60 min, thorough wash in tap water, and sterilization by placing in 70% ethanol for 12 h. Before use, filters were soaked in sterile phosphate buffered saline and tacked to the bottom of the culture dish with a soldering pencil. Freshly trypsinized LLC-PK_1 cells were plated onto the filters and grown in Minimal Essential Medium (GIBCO) supplemented with 10% porcine serum, 10 μg/ml gentamycin and 5 μg/ml insulin. Cultures were kept in a 37 °C incubator gassed with 5% CO_2 and 95% air and were ready for use in about one week.

Electron Microscopy

Preparation for transmission electron microscopy was as described (Pickett et al., 1975).

Experimental Chamber

All electrical and flux measurements were carried out in an Ussing chamber. The filter preparation was carefully removed from the culture dish and placed between halves of the chamber, sandwiched between two silicone washers. The exposed filter area was 2.54 cm^2. Each chamber half contained a well to accommodate a small magnetic stir bar; the fluid volume was 8 ml per side. Platinum-irridium wire was used to pass current through the chamber and potential difference measurements (PD) were made with a pair of Ag-AgCl electrodes contained in a microelectrode holder (W.P. Instruments). A 5-cm length of polyethylene tubing (PE50) filled with 3 M KCl in 3% agar was placed within 2 mm of the filter surface. Both current passing and potential measuring bridges were input to an automatic voltage clamp (D. Lee Instruments, Sunnyvale, Calif.). The output from the clamp was fed into a two-channel Varian recorder (G2000). In the voltage-clamp mode, the tissue is continuously short circuited except for a 3-sec period each minute to measure open-circuit voltage. Tissue resistance was calculated from Ohm's law. The Ussing chamber was immersed in a 37 °C water bath which sits atop a magnetic stirrer. Solution in the chamber was changed by a Harvard infusion pump and the filter preparation maintained under solution with no hydrostatic pressure difference between chamber halves. Electric measurements were stable over several hours, although frequent solution changes were associated with a gradual decrease in resistance.

Short-Circuit Current vs. [Sugar]

Sugar and other compounds were tested for their ability to stimulate short-circuit current (SCC) by the addition of aliquots from a 50-mM solution. An Eadie-Hofstee plot of SCC *vs.* SCC/[sugar] allowed calculation of an assumed relative affinity of the nonelectrolyte to the carrier mechanism and an apparent maximal SCC stimulation. In this type of analysis, the slope of the graph is given as $-K_{\frac{1}{2}}$ and the y intercept is SCC_{max}. The effect of an osmotically induced streaming potential due to solute addition was disregarded as up to a 50-mM mannitol gradient was associated with <0.2 mV change in potential.

Transepithelial Sugar Fluxes

Simultaneous bidirectional sugar fluxes using ^{14}C and ^{3}H forms of glucose (New England Nuclear) measured the amount of net glucose movement (usually 1 μCi ^{14}C glucose to the apical solution, 10 μCi ^{3}H glucose to basolateral). Fifty μl bath samples were taken every 15 min, beginning after a 3-min isotope equilibration period. Samples were counted in a dual channel scintillation counter (Beckman LS100), along with a ^{14}C-glucose sample to correct for ^{14}C counts counted by the ^{3}H channel. Unidirectional fluxes were calculated according to the equation:

$$FLUX_{12} = \frac{cpm_{t2} - cpm_{t1}}{X}$$

where the flux from compartment 1 to 2 is equal to the difference in count rate between successive 15-min samples in compartment 2 divided by X, the specific activity of sugar (cpm/μmol) of compartment 1. Following corrections for time and surface area, results are expressed as μmol h^{-1} cm^{-2}. Net flux is the difference between apical and basolateral unidirectional fluxes.

Glucose as a Tracer

The question of whether glucose can be used as a transepithelial tracer without appreciable loss of label or metabolism was checked in the following manner. A unidirectional ^{14}C glucose apical-to-basolateral flux experiment was run for 60 min, and samples from both the apical and basolateral compartments placed on a silica gel thin layer plate and eluted in an ascending chromatography tank with (50:32:18, butanol/ethanol/water). After drying, the plate was placed against X-ray film and exposed for several weeks. The position of the resulting spots was compared to a ^{14}C D-glucose control.

Solutions

Hanks' salt solution (HSS), pH 7.2, used in most of the experiments had the following composition (mM):

NaCl,	140.0;	$NaHCO_3$	4.2;	$MgCl_2$,	0.5;
Na_2HPO_4	0.36;	HEPES	10.0;	KCl	5.4;
$CaCl_2$	1.3;	KH_2PO_4	0.44;	Glucose	5.5.

For testing other sugars, glucose was left out of the formulation. Na-free HSS was made with either choline or Tris. Adjustments in pH were made with NaOH with the respective K salts replacing those of Na when necessary.

Chemicals

All sugars and phlorizin were obtained from Sigma Chemical Company.

Results

A. Morphology

When LLC-PK_1 cells are cultured on a collagen-coated Millipore filter (MF), sections for transmission electron microscopy perpendicular to the epithelial sheet demonstrated well-defined microvilli (MV) localized to the apical (brush border) surface extending into the medium (Fig. 1). Circumferential tight junction (Tj) complexes join adjacent cells into a continuous sheet and the cells form a monolayer resting on the collagen-coated Millipore filter. From our investigations, it is not possible to comment on the asymmetry of intracellular organelles.

B. Electrical Properties

A transepithelial potential difference (PD) and short-circuit current (SCC) were effected by the cell layer

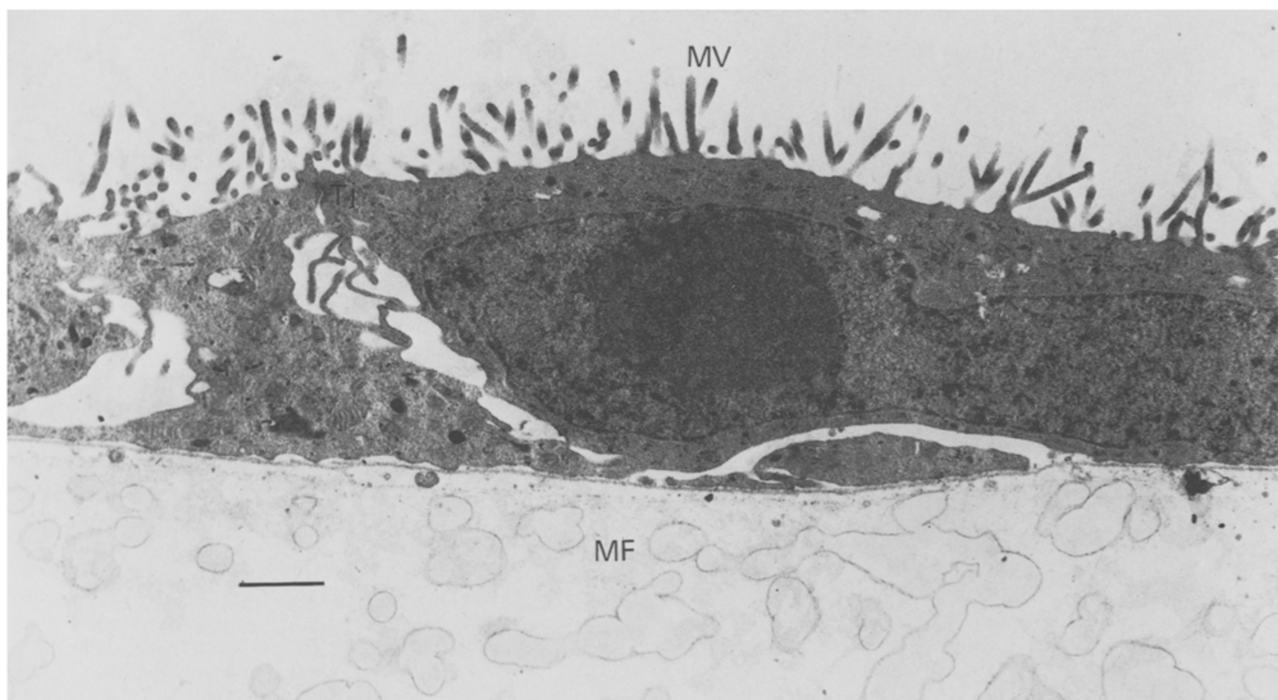

Fig. 1. Transmission electron micrograph of kidney cell layer sectioned perpendicular to plane of the sheet of cells. The cellular monolayer rests on a film of rat tail collagen which was applied to the methyl cellulose membrane filter (*MF*). Microvilli (*MV*) extend from the apical cell surface into the medium and a juxta-apical tight junction (*Tj*) joins adjacent cells. Bar equals 1 μM

Table 1. Basic transepithelial electrical properties of LLC-PK_1 monolayer

Potential difference (mV)	$-2.7\pm$ 0.16 (32)[a]
Short-circuit current (μA cm^{-2})	$13.0\pm$ 0.7 (32)
Resistance (ohm · cm^2)	211.0 ± 13.0 (44)

[a] $\bar{x}\pm$SEM(n) measured at 37 °C in HSS (contains 5.5 mM glucose).

when placed in an Ussing chamber with identical Hanks' salt solution (HSS) on either side (Table 1). Collagen coated Millipore filters without cells and multilayered mouse fibroblasts show minimal resistance (6 ohm · cm^2) and no observable PD or SCC.

In the absence of D-glucose, a minimal SCC of 1.2 μA cm^{-2} was promptly increased an order of magnitude on the addition of D-glucose to the apical bath (Fig. 2). Added D-glucose caused the PD to increase from -0.46 mV ± 0.06 (11) to -2.7 ± 0.16 (32) with no significant decrease in resistance. Apical addition of phlorizin (2.5×10^{-5} M) resulted in a prompt 85% reduction in SCC.

Progressive amounts of D-glucose resulted in a saturable SCC and PD response (Fig. 3). Sugars which stimulate SCC were analyzed by an Eadie-Hofstee plot of SCC *vs.* SCC/[sugar] (Fig. 4). This allowed determination of the sugar concentration required for half-maximal stimulation of SCC ($K_{\frac{1}{2}}$) and gave a value for comparing the relative affinity of

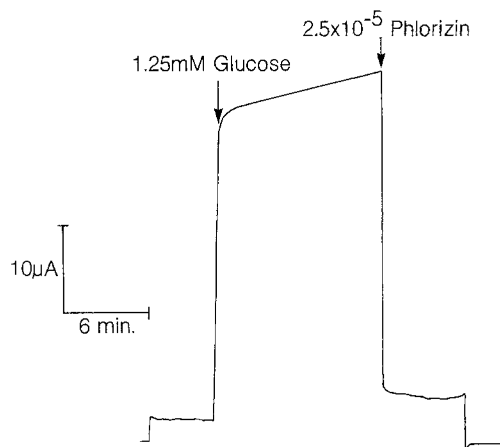

Fig. 2. The effect of D-glucose and phlorizin on SCC when added to a D-glucose-free bathing medium. The cell sheet has been placed in an Ussing chamber at 37 °C, the voltage clamped to zero, and SCC measured

sugars to the transport mechanism (Table 2). The calculated SCC_{max} for D-galactose, α-methyl-D-glucoside, and 3-O-methyl-D-glucose was not statistically different from D-glucose although for β-methyl-D-glucoside and 6-deoxy-D-glucose the SCC_{max} was significantly less. Other sugars and compounds with reported Na-dependent transport were evaluated by addition to glucose-free HSS at concentrations of 1–2 mM for their ability to stimulate SCC and none was detected (Table 3).

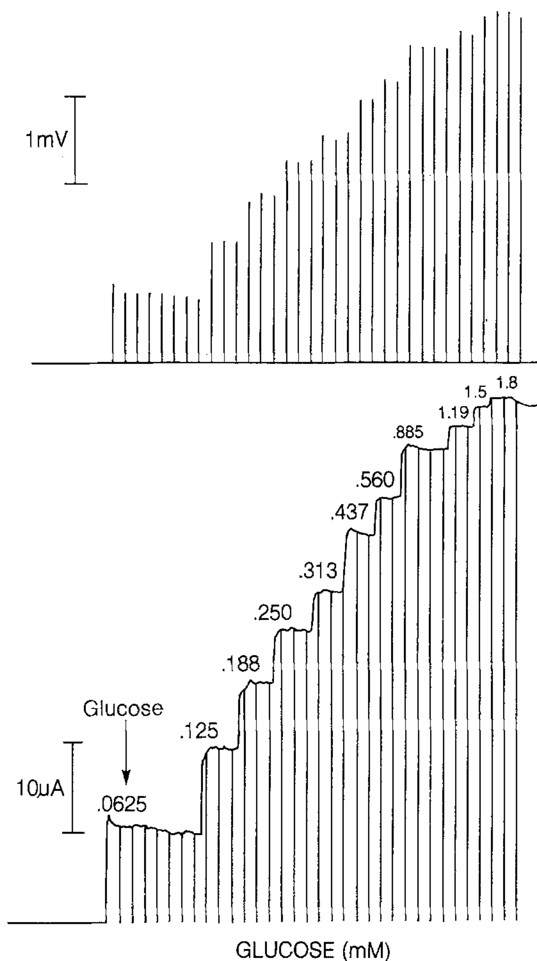

Fig. 3. Increasing amounts of D-glucose in the apical bath stimulates SCC and transepithelial PD in a saturable fashion. The cell layer was placed in an Ussing chamber, and an automatic voltage clamp allowed continuous recording of SCC (lower tracing) except for a 3-sec period each minute when the open-circuit voltage is measured (upper tracing)

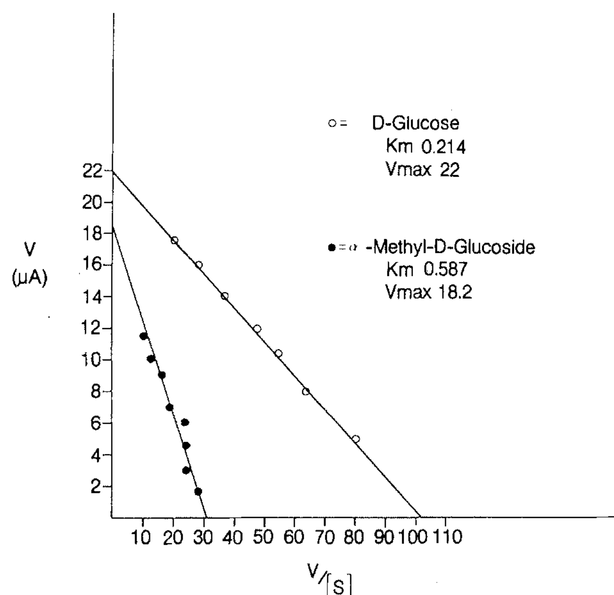

Fig. 4. An Eadie-Hofstee plot of a typical experiment analyzing the effect of sugar concentration on SCC for D-glucose and α-methyl-D-glucoside. The slope and intercept were determined by linear regression

Table 2. Concentration of sugars to stimulate SCC ability

	$K_{\frac{1}{2}}$ (mM)	V_{max}^{SSC} (μA cm^{-2})
β-methyl-D-glucoside	0.131±0.01 (7)[b]	4.9±1.1 (7)[b]
D-glucose	0.284±0.03 (17)	11.7±1.9 (17)
α-methyl-D-glucoside	0.653±0.04 (7)[b]	8.4±2.1 (7)[a]
6-deoxy-D-glucose	0.772±0.08 (8)[b]	2.3±0.14 (8)[b]
D-galactose	4.76 ±1.4 (4)[b]	15.1±3.8 (4)[a]
3-O-methyl-D-glucoside	29.5 ±3.0 (4)[b]	17.1±6.4 (4)[a]

Paired t-test to D-glucose.
[a] $P>0.05$.
[b] $P<0.05$.
$\bar{x}\pm$SEM(n)

Table 3. Compounds unable to stimulate SCC

L-glucose	Sucrose
D-xylose	L-alanine
D-fructose	L-proline
2-deoxy-D-glucose	Citrate
Myoinositol	Succinate
α-aminoisobutyrate	D-mannose

Table 4. Effect of sodium concentration on D-glucose-dependent SCC

Na (mM)	$K_{\frac{1}{2}}$ (mM)	V_{max}^{SCC} (μA cm^{-2})
140	0.30±0.02 (12)	13.8±1.2 (12)
70	0.51±0.05 (6)[a]	12.8±1.7 (6)
20	1.30±0.25 (6)[a]	9.7±1.4 (6)[a]

$\bar{x}\pm$SEM(n).
[a] Paired t-test $P<0.05$ compared to 140 mM Na.

Effect of Sodium on D-glucose Dependent SCC

Reduction of the sodium concentration by substitution with Tris from 140 to 70 mM resulted in a 70% increase in the amount of D-glucose for half-maximal SCC, but no significant change in maximal SCC. However, further reduction to 20 mM Na produced a 4.3-fold increase in $K_{\frac{1}{2}}$ and was associated with a 30% reduction in maximal SCC (Table 4). Thus over the range of Na concentrations chosen, the effect of decreased Na on $K_{\frac{1}{2}}$ is more pronounced than on maximal SCC.

Transepithelial Flux of Isotopically Labeled D-Glucose

The validity of isotopically labeled D-glucose requires that the label remain intact and unmodified by metabolism. The flux of ^{14}C and ^{3}H labeled D-glucose was simultaneously measured during the apical-to-basola-

Table 5. Simultaneous $^{3}H + ^{14}C$ glucose apical to basolateral flux

Period	^{3}H	^{14}C
1	0.050[a]	0.057
2	0.116	0.109
3	0.061	0.066

[a] μmol cm^{-2} h^{-1}

Table 6. $^{14}C + ^{3}H$ bidirectional simultaneous D-glucose flux (μmol cm^{-2} h^{-1})

Direction	Control	Phlorizin
$J_{A \to Bl}$	0.259±0.02 (10)	0.130±0.02 (4)
$J_{Bl \to A}$	0.150±0.03 (10)	0.133±0.04 (4)

$\bar{x}$±SEM(n) at 37 °C in HSS (contains 5.5 mM glucose).

teral transport (Table 5). The equivalent transport rates over sequential 15-min periods suggest no significant metabolism of the labeled glucose and that the labeled molecules have not followed separate metabolic paths. Autoradiographic analysis of chromatographed ^{14}C-labeled D-glucose before and after apical-to-basolateral transport gave identical elution patterns without detectable migration of labeled compounds other than D-glucose. Net apical-to-basolateral D-glucose flux was 0.1 μmol cm^{-2} h^{-1} by simultaneous bidirectional fluxes of ^{14}C and ^{3}H labeled D-glucose. Phlorizin (10^{-5} M) added to the apical bath eliminated the net transport by reducing the apical-to-basolateral flux to that of basolateral-to-apical (Table 6).

Discussion

Transepithelial D-glucose transport by the pig kidney cell line LLC-PK_1 provides a model for D-glucose transport by the proximal nephron or small intestine. The epithelial monolayer is able to effect net D-glucose transport that is sodium dependent and phlorizin inhibited with an associated and saturable SCC as D-glucose concentration is increased. D-glucose analogs stimulate SCC with a similar hierarchy of effectiveness as observed in small intestine (Crane, 1960; Barry, Smyth & Wright, 1965) and rat proximal tubule (Ullrich, Rumrich & Kloss, 1974). The structural requirements of glucose analogs capable of stimulating SCC across the LLC-PK_1 cell lines is also the same pattern associated with cellular depolarization (Fromter, 1979) and steady-state concentration differences (Ullrich et al., 1974) across rat proximal tubule. In each case, the configuration requirement for D-glucopyranosides and the D-glucose equatorial position of C2 OH group was absolute with less stringent requirements for the OH at C6 or the α or β plane of a methyl group at Cl.

The saturable response of SCC to increasing sugar concentrations allows estimation of the assumed glucose carrier affinity by the concentration required for half-maximal SCC ($K_{\frac{1}{2}}$). Our determination of $K_{\frac{1}{2}}$ for D-glucose of 0.28 mM is comparable to 0.2 mM for the high affinity site in dog kidney brush border vesicles (Turner & Silverman, 1978), 0.125–0.135 mM for rabbit proximal tubule brush border membrane in reconstituted liposomes (Fairclough et al., 1979) and 1.0 mM from the relation to membrane potential in rat proximal tubule (Fromter, 1979). The SCC maximum for D-glucose, α-methyl-D-glucoside, 3-O-methyl-D-glucose and D-galactose was not significantly different despite the 100-fold range in $K_{\frac{1}{2}}$; however, the SCC maximum for 6-deoxy-D-glucose and β-methyl-O-glucoside were significantly less.

In the mammalian ileum (Schultz & Zalusky, 1964; Barry et al., 1965), glucose-dependent Na flux was directly measured and equivalent to SCC. Effect of D-glucose on transepithelial SCC determinations and solute transport have not been performed on renal tissues; however, Burg et al. (1976) described increased proximal tubule fluid and presumably Na absorption when D-glucose was added to the perfusate. Our studies (to be published) also demonstrate an equivalence between net Na transport and SCC.

The effect of reduced sodium on D-glucose-stimulated SCC was to decrease the assumed affinity to the carrier with an increase in the concentration of D-glucose required for half-maximal SCC (Table 4). Other reports describe a direct relationship between the concentration of Na and the affinity of phlorizin and D-glucose binding to isolated renal apical membranes (Frasch et al., 1970; Chesney, Sacktor & Kleinzeller, 1974). Although apical membrane binding may be the limiting step, our experiments do not address the site or primary effect of Na for D-glucose-stimulated SCC.

In addition to those sugars not activity transported (Ullrich et al., 1974), amino acids associated with Na-dependent apical membrane depolarization (Fromter, 1979) or tricarboxylic acid cycle intermediates which show Na-dependent apical vesicle accumulation (Kippen et al., 1979) were not able to stimulate SCC (Table 4). Studies of depolarization of rat proximal tubule apical membrane by Samarzija and Fromter (*summarized by* Ullrich, 1979) suggested interaction among Na-dependent sugar and amino acid transport systems without delineation of the mechanism responsible. Whether a single cell type transports a single Na-dependent solute and LLC-PK_1 represents a clone dedicated to sugar transport

or whether there has been a loss of other transport systems during cell culture is not possible to discern.

The transported D-glucose is not detectably metabolized by the LLC-PK_1 as evidenced by the identical elution pattern of labeled D-glucose before and after transport. Isotopically labeled ^{14}C glucose introduced in the renal artery was recovered intact from the renal vein with no nonvolatile radioactivity other than glucose and 0.06% as $^{14}CO_2$ (Chinard et al., 1959). Minimal decrease in glucose recovery was demonstrated during glucose transport studies on isolated perfused rabbit proximal tubule (Tune & Burg, 1971). Not only is there no evidence for glucose metabolism as a consequence of proximal tubule transport, there is little evidence for renal glucose metabolism at all (Cohen & Barac-Nieto, 1973).

D-glucose transport by the LLC-PK_1 cells has several features that fulfill criteria as an ideal system for detailed study of the mechanism of transepithelial solute transport. LLC-PK_1 is a homogeneous population of epithelial cells that form a functional epithelial tissue, a simple monolayer sheet devoid of other cellular elements. The transported solute is biologically important, yet investigation of its fate not complicated by interaction with cellular metabolism. Net transport is distinguishable from bidirectional flux and is electrogenic. The cell culture system provides an additional unique advantage, that of the genetic approach to biochemical mechanisms. This kidney cell line can be cloned from a single cell which allows for the potential to select populations of unique mutants for D-glucose transport (Puck, 1972).

The research was supported in part by the Cobb Foundation, Dufrense Foundation, the Veterans Administration and Grant No. 5 T32 CA09287 awarded by the National Cancer Institute, Department of Health, Education and Welfare.

References

Barry, R.J.C., Smyth, D.H., Wright, E.M. 1965. Short-circuit current and solute transfer by rat jejunum. *J. Physiol. (London)* **181**:410

Bisbee, C.A., Machen, T.E., Bern, H.A. 1979. Mouse mammary epithelial cells on floating collagen gels. Transepithelial ion transport and effect of prolactin. *Proc. Natl. Acad. Sci. USA* **76**:536

Burg, M., Patlak, C., Green, N., Villey, D. 1976. Organic solutes in fluid absorption by renal proximal convoluted tubules. *Am. J. Physiol.* **231**:627

Cereijido, M., Robbins, E.S., Dolan, W.J., Rotunno, C.A., Sabatini, D.D. 1978. Polarized monolayers formed by epithelial cells on a permeable and translucent support. *J. Cell Biol.* **77**:853

Chesney, R., Sacktor, B., Kleinzeller, A. 1974. The binding of phloridizin to the isolated luminal membrane of the renal proximal tubule. *Biochim. Biophys. Acta* **332**:263

Chinard, F.P., Taylor, W.R., Nolan, M.F., Enns, T. 1959. Renal handling of glucose in dogs. *Am. J. Physiol.* **196**:535

Cohen, J.J., Barac-Nieto, M. 1973. Renal metabolism of substrates in relation to renal function. *In:* Handbook of Physiology. Section 8: Renal Physiology. J. Orloff and R.W. Berliner, editors. p. 964. American Physiological Society, Washington, D.C.

Crane, R.K. 1960. Intestinal absorption of sugar. *Physiol. Rev.* **40**:789

Fairclough, P., Malathi, H., Presier, H., Crane, R.K. 1979. Reconstitution into liposomes of glucose active transport from the rabbit renal proximal tubule. Characteristics of the system. *Biochim. Biophys. Acta* **553**:295

Frasch, W., Frohnert, P.P., Bode, F., Baumann, K., Kinne, R. 1970. Competitive inhibition of phlorizin binding by D-glucose and the influence of sodium: A study on isolated brush border membrane of rat kidney. *Pflueger's Arch.* **320**:265

Fromter, E. 1979. Solute transport across epithelia: What can we learn from micropuncture studies on kidney tubules? *J. Physiol. (London)* **288**:1

Hull, R.N., Cherry, W.R., Weaver, G.W. 1976. The origin and characteristics of a pig kidney cell strain LLC-PK_1. *In Vitro* **12**:670

Kippen, I., Hirayama, B., Klinenberg, J.R., Wright, E.M. 1979. Transport of tricarboxylic acid cycle intermediates by membrane vesicles from renal brush border. *Proc. Nat. Acad. Sci. USA* **76**:3397

Leighton, J., Brada, Z., Estes, L.W., Justa, G. 1969. Secretory activities and oncogenicity of a cell line (MDCK) derived from a canine kidney. *Science* **163**:472

Misfeldt, D.S., Hamamoto, S.T., Pitelka, D.R. 1976. Transepithelial transport in cell culture. *Proc. Nat. Acad. Sci. USA* **73**:1212

Mullin, J.M., Diamond, L., Kleinzeller, A. 1979. Uptake of alpha-methyl-D-glucoside and 3-O-methyl-D-glucose by an established pig renal epithelial line. *Fed. Proc.* **38**:1058

Owens, R.B., Smith, H.S., Hacket, A.J. 1974. Epithelial cell culture from normal glandular tissue of mice. *J. Nat. Cancer Inst.* **53**:261

Pickett, P.B., Pitelka, D.R., Hamamoto, S.T., Misfeldt, D.S. 1975. Occluding junctions and cell behavior in primary cultures of normal and neoplastic mammary-gland cells. *J. Cell Biol.* **66**:316

Puck, T.T. 1972. The Mammalian Cell as a Microorganism. Holden-Day, San Francisco

Rabito, C.A., Ausiello, D.A. 1980. Na^+-dependent sugar transport in a cultured epithelial cell line from pig kidney. *J. Membrane Biol.* **54**:31

Schultz, S.G., Zalusky, R. 1964. Ion transport in isolated rabbit ileum. I. Short-circuit current and Na fluxes. *J. Gen. Physiol.* **47**:567

Taub, M., Chuman, L., Saier, M.H., Sato, G.H. 1979. The growth of a kidney epithelial cell line (MDCK) in hormone-supplemented serum-free media. *Proc. Nat. Acad. Sci. USA* **76**:3338

Tune, B.M., Burg, M.B. 1971. Glucose transport by proximal renal tubule. *Am. J. Physiol.* **221**:580

Turner, R.J., Silverman, M. 1978. Sugar uptake into brush border vesicles from dog kidney. II. Kinetics. *Biochim. Biophys. Acta* **511**:470

Ullrich, K.J. 1979. Sugar, Amino acid and Na cotransport in proximal tubule. *Ann. Rev. Physiol.* **41**:181

Ullrich, K.J., Rumrich, G., Kloss, S. 1974. Specificity and sodium dependence of the active sugar transport in the proximal convolution of the rat kidney. *Pfluegers Arch.* **351**:35

Received 16 May 1980; revised 17 July 1980