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Transepithelial Transport in Cell Culture: D-Glucose Transport by a Pig Kidney Cell Line (LLC-PK$_1$)

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Summary. The pig kidney cell line LLC-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 ^{3}H 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-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).

Materials and Methods

Cell–Filter Preparation

Pig kidney cell line LLC-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

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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:

$$\mathrm{FLUX}_{12}=\frac{\mathrm{cpm}_{t2}-\mathrm{cpm}_{t1}}{X}$$

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