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Report

The Madin Darby Canine Kidney (MDCK) Epithelial Cell Monolayer as a Model Cellular Transport Barrier

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Two strains of Madin Darby canine kidney (MDCK) cells were grown on a polycarbonate membrane with 3-μm pores without any extracellular matrix treatment. The membrane, 2.45 cm in diameter, which is part of a commercially obtained presterilized culture insert, provides two chambers when placed in a regular six-well culture plate. This device was found to be convenient for investigating transport of a few selected fluid-phase markers across the MDCK cell monolayer. Both the strain from the American Type Culture Collection (ATCC) and the so-called highly resistant strain I, at a serial passage between 65 and 70, showed a seeding concentration-dependent lag phase followed by a growth phase with a 21-hr doubling time. When seeded at 5×10^4 cells/cm^2, cell confluence was achieved in 5 days in a modified Eagle's minimum essential medium (MEM) containing 10% fetal bovine serum under a 5% CO_2 atmosphere. Similarly, transepithelial electrical resistance (TEER) also reached a plateau value in 5 days. Both light and electron microscopic examinations revealed well-defined junctional structures. Transport of the fluid-phase markers, sucrose, lucifer yellow CH (LY), inulin, and dextran across the MDCK cell monolayers was studied primarily at 37°C following the apical-to-basolateral as well as the basolateral-to-apical direction. Large variations in the steady-state transport rate were observed for a given marker between the cell layer preparations. Thus, the present study proposes an "internal standard" procedure for meaningful comparisons of the transport rate. When normalized to the rate of sucrose, the rate ratio was 1.00:0.80:0.67:0.15 for sucrose:LY:inulin:dextran. This ratio was virtually independent of temperature, cell strain, direction of the marker migration, and TEER value, suggesting a common transport mechanism. The observed rate ratio appears to reflect molecular size and charge. The transport observed in the present study would consist, in theory, of both paracellular shunt and transcellular vesicular transport. Quantitative assessment of each transport mechanism in the overall transport has been difficult. The initial uptake of [^{3}H]dextran estimated for the slowest transport observed in the present study was still 300-fold faster than a literature value. This appears to indicate that the transport observed in the present study is largely through the paracellular shunt pathway.

KEY WORDS: Madin Darby canine kidney (MDCK) epithelial cell monolayer; model cellular transport barrier; fluid-phase markers; transcellular vesicular transport; paracellular shunt; transepithelial electrical resistance.

INTRODUCTION

Unless proven otherwise, transport of drug substances across various cellular barriers *in vivo* is assumed to occur via a nonspecific diffusional process governed solely by a concentration gradient. Under this condition, physicochemical properties of the diffusing solute molecule and the physiological function of the cell layer involved are the important factors dictating the transport rate. For polar substances which do not partition onto the cell membrane, endocytic fluid-phase pinocytosis (transcellular) and leakage through the intercellular lateral space (paracellular) should be the major transport pathways. For substances with a substantial lipid solubility, the partition onto the cell membrane would be followed by envagination/vesiculation of the membrane (adsorptive pinocytosis) and/or non-energy-requiring lateral diffusion within the cell membrane (1,2). An experimental model that can be used in sorting out these potential transport mechanisms is highly desirable for substances that are not subject to facilitated transports. It would allow assessment of the effect of physicochemical properties on the transport in a more quantitative manner at a cellular and molecular level. The ultimate outcome would be a rational drug design in which the transport property is optimized.

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Towards this end, we decided to study the transport of a few selected fluid-phase markers across the Madin Darby canine kidney (MDCK)[2] epithelial cell monolayer. Cellular uptake, as part of transcellular transport, of these markers would occur only via pinocytosis.

The MDCK cell line is perhaps one of the best-characterized epithelial cell lines easily available for a disparity of studies in cell biology. It was originally derived from the kidney of a normal male cocker spaniel in 1958. When grown on a microporous membrane, confluent cell monolayers are formed with well-developed intercellular occluding junctions (3,4), thus providing a convenient model for studying transepithelial transport. With certain limitations, the system may well be adopted as a model for studying the drug transport across renal, gastrointestinal, intranasal, rectal, and other epithelia in the body. At least two different strains of MDCK cells have been reported in the literature: strain I forms a "tight" epithelium with transepithelial electrical resistances (TEER) above 1000 $\Omega \cdot cm^2$ (5,6), while strain II forms a "leaky" epithelium with TEER of the order of 100 $\Omega \cdot cm^2$ (5,7). Both cell lines have been extensively studied in conjunction with transport of substances that are normally subject to renal functions, in particular ion transport (3,4,6,8). To our knowledge, however, the system has not been employed for transport studies on solutes of pharmaceutical interest. Most of the studies reported in the literature used cell monolayers grown over cellulose-based (9,10) or polycarbonate (11) membranes of different pore sizes with or without various extracellular matrices. In recent years, presterilized culture inserts have become commercially available for both types of membrane; for example, the Millicell system, using a cellulose membrane (Millipore Corp., Bedford, Mass.), and the Transwell system, based on a polycarbonate membrane (Costar Corp., Cambridge, Mass.), have further facilitated the use of MDCK cell monolayers. In the present study, two strains of MDCK cells were grown in the 3-μm Transwell system without any matrix treatment. The transport of the fluid-phase markers was monitored both in the basolateral-to-apical and in the apical-to-basolateral direction. The markers used are lucifer yellow CH(LY) (12), sucrose, inulin (9), and dextran.

MATERIALS AND METHODS

Materials. The original MDCK cell line was obtained from the American Type Culture Collection (ATCC), Rockville, Md., at serial passage 52. High-resistant strain I at serial passage approximately 60 was from Dr. N. L. Simmons (University of St. Andrews, Scotland) via Dr. G. K. Ojakian (State University of New York at Brooklyn). Normal and Ca^{2+}/Mg^{2+}-free Hanks' balanced salt solution (10×) and a powder form of Eagle's minimum essential medium (MEM) with Earle's salts and L-glutamine were obtained from Gibco Laboratories (Grand Island, N.Y.). Medium at normal strength was prepared following the manufacturer's recommended procedure. Fetal bovine serum from HyClone Laboratories (Logan, Utah) was heat-inactivated in a water bath at 56°C for 30 min after being thawed at room temperature. A penicillin/streptomycin mixture, at 5000 U/ml and 5 mg/ml, respectively, and a trypsin/EDTA mixture, 1:250 (1×), were obtained from Flow Laboratories (McLean, Va.). The Transwell, PVP-free, of 24.5-mm diameter and 3.0-μm pore size, was obtained from Costar (Cambridge, Mass.). Other plasticware for standard cell culture was from commercial sources such as Fisher Scientific (Pittsburgh, Pa.) and Nalge Co. (Rochester, N.Y.). The medium for MDCK cell culture was routinely prepared by mixing 900 ml of the above Eagle's MEM, 100 ml of heat-inactivated fetal bovine serum, 10 ml of the antibiotic solution, and 10 mmol of each of *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES) and its sodium salt (U.S. Biochemical Corp., Cleveland, Ohio) and sterilizing using 0.2-μm filtration. This mixture is referred to simply as medium hereafter. Similarly normal and Ca^{2+}/Mg^{2+}-free Hanks' balanced salt solutions used in the present study contained 20 m*M* HEPES. These solutions are referred to as HBSS and CMF/HBSS, respectively.

Lucifer yellow CH (LY) was obtained from Molecular Probes (Eugene, Ore.) and used without further purification. [^{14}C]Sucrose and [^{3}H]inulin (MW 5000–5500) were obtained from New England Nuclear (Boston, Mass.), whereas [^{3}H]dextran (MW 50,000–125,000) was from Amersham (Arlington Heights, Ill.). The latter two radionuclides were purified using a Sephadex G-100 (Pharmacia, Piscataway, N.J.) column and HBSS as an eluent just prior to use. Cold inulin and sucrose were obtained from Sigma (St. Louis, Mo.) and Mallinckrodt (Paris, Ky.), respectively.

Cells. MDCK cells were thawed from a mixture of 10% dimethyl sulfoxide and 20% heat-inactivated fetal bovine serum in HBSS at −135°C. They were cultured in plastic T-flasks using the medium defined earlier under a 5% CO_2 atmosphere at 37°C. The medium was changed every other day. At approximately 70% confluence, cells were washed twice with CMF/HBSS and rounded up after incubation at 37°C for 7–10 min in a minimum volume of the trypsin/EDTA mixture. They were suspended in CMF/HBSS such that 0.1 ml contained a sufficient number of cells for a given cell seeding density. Cell pellets, whenever needed, were routinely obtained by centrifugation at 50*g* for 5 min. Cell concentration was determined after proper dilution in a saline solution (Isoton II, Curtin Matheson Scientific, Houston, Tex.) using a Coulter counter Model ZM cell counter (Hialeah, Fla.).

Into each of six-well plates of 35-mm diameter (Costar), 2.60 ml of the medium was first added. Transwells with the membrane surface area 4.71 cm^2 were then placed, into which 1.40 ml of the medium was introduced. Cells were added in 0.10-ml aliquots in CMF/HBSS at a desired cell concentration, ranging from 0.5 to 5.0 × 10^4 cells/cm^2. From day 2, the medium was changed every day. For both strains of MDCK cells, the seeding was made at a serial passage between 60 and 70. Cell density at a given time was determined using the Coulter counter Model ZM after the cells were rounded up as follows. After a brief wash in saline in a beaker, a Transwell was incubated at 37°C for 25–30 min

[2] Abbreviations used: ATCC, American Type Culture Collection; CMF/HBSS, Ca^{2+}/Mg^{2+}-free HBSS; HBSS, Hanks' balanced salt solution; Hepes, *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid; LY, lucifer yellow CH; MDCK, Madin Darby canine kidney; MEM, minimum essential microscopy; SEM, scanning electron microscopy; TEER, transepithelial electrical resistance; TEM, transmission electron microscopy.

with 2.6 and 1.4 ml of the trypsin/EDTA mixture in the well and the cup, respectively. Cells were detached from the membrane support through rapid trituration with a Fisherbrand Transfer Pipet (Fisher Scientific, Pittsburgh, Pa.). The medium containing cells was quantitatively combined and diluted in Isoton II. The total number of cells recovered was divided by 4.71 cm^2.

Cell morphology was examined under a light microscope following normal glutaraldehyde fixation and hematoxylin/eosin staining steps. Since the polycarbonate membrane with 3.0-μm pores is not translucent, the following procedure was adopted. After the stained cell monolayer is dried, it is cut out of the well frame. The sample is then placed on a microscope slide with the aid of a few drops of chloroform, with the face of the cell layer down. Once the solvent is evaporated, the cell layer generally adheres to the slide surface nicely. The slide is then immersed in a Coplin jar of chloroform for 15–20 min to dissolve the synthetic membrane gently.

Transepithelial Electrical Resistance (TEER) Measurement. Electrical resistance across MDCK monolayers was measured using the four-electrode technique (13). A Transwell containing a cultured monolayer was inserted directly into an acrylic chamber designed specifically for their accommodation (Fig. 1: manufactured by Laboratory Automation Support, The Upjohn Co.). Electrodes made from 1.2-mm glass capillary tubing were filled with 2.0 *M* NaCl plus 3% agar and connected to AG/AgCl pellet electrodes (World Precision Instruments, New Haven, Conn.). Voltage asymmetry between these electrodes was less than 1 mV. Direct-current pulses were delivered through one pair of electrodes, positioned 5 mm above and below the monolayer, by way of a stimulator connected to stimulus isolation and constant-current units (Grass Instruments, Quincy, Mass.) Voltage changes in response to injected current were detected by way of a second pair of electrodes, positioned 1 mm above and below the monolayer, that were connected to a high-impedance differential amplifier (World Precision Instruments). Current and voltage signals were observed on an oscilloscope (Tektronix Instruments, Beaverton, Ore.) and stored on a chart recorder (Gould Electronics, Cleveland, Ohio). In the present study, TEER measurements were initiated 3–4 days after the seeding of cells and continued until cell growth curves plateaued or until transport studies were completed. Preliminary studies revealed that monolayers formed from both ATCC and strain I cells are characterized by a linear *I/V* relationship over a wide range of current values. TEER values for each monolayer were derived using Ohm's law ($V = IR$) from the mean voltage change recorded in response to 8–10 hyperpolarizing current pulses of 500-msec duration. Resistance values thus derived were multiplied by 4.71 cm^2 to account for the surface area of the polycarbonate membrane supporting the cells. For each series of measurements, background resistance was determined using several unseeded wells, and the mean values obtained (25–30 $\Omega \cdot cm^2$) were subtracted from each experimental well.

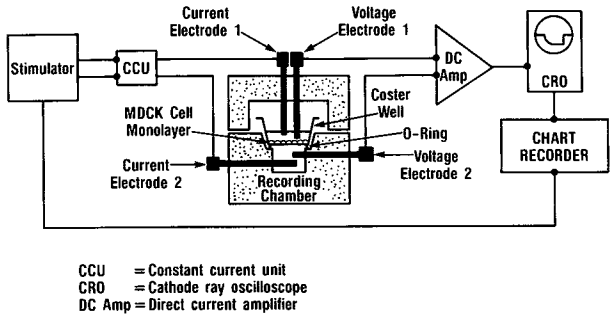

Fig. 1. Schematic diagram of the method by which TEER was measured.

Transport Studies. Upon cell confluence on the polycarbonate membrane, which is usually achieved in 5 days, the medium was replaced with HBSS. After TEER measurement, the cell monolayers in HBSS were further equilibrated at either 4 or 37°C for at least 30 min. In protocol 1, transport of fluid-phase markers was monitored in the direction of basolateral to apical domains. In protocol 2, the transport was studied in the opposite direction. Solution 1 in HBSS contained approximately 5×10^6 dpm/ml of [^{14}C]sucrose, 0.5 mg/ml of LY, and 3×10^7 dpm/ml of [^{3}H]inulin. Solution 2 contained, in addition to sucrose and LY, 2×10^7 dpm/ml of [^{3}H]dextran. Stock solutions in HBSS were equilibrated at either 4 or 37°C before the transport study began. In some studies cold materials were also added to the stock solutions to adjust the final solute concentration to 1.0 m*M*. No significant difference in transport was noticed.

The study in protocol 1 was carried out in one well. At a given time interval, 1.5 ml of HBSS in the receiving cup was replaced with 1.5 ml of fresh HBSS at 37 or 4°C. Care was taken not to damage the cell monolayer with the tip of a Fisherbrand Transfer Pipet. In the case of protocol 2, the donor cup was simply moved at a given time interval to an adjacent well containing 2.6 ml of fresh HBSS. The concentration of LY in the receiving compartment was determined from fluorescence intensity measured on an SLM/Aminco Model SPF-500 spectrofluorometer (SLM/Aminco, Urbana, Ill.) with excitation at 430 nm (bandpass, 10 nm) and emission at 540 nm (bandpass, 20 nm). The concentration term was converted to mass units per 1.5 or 2.6 ml and the accu-

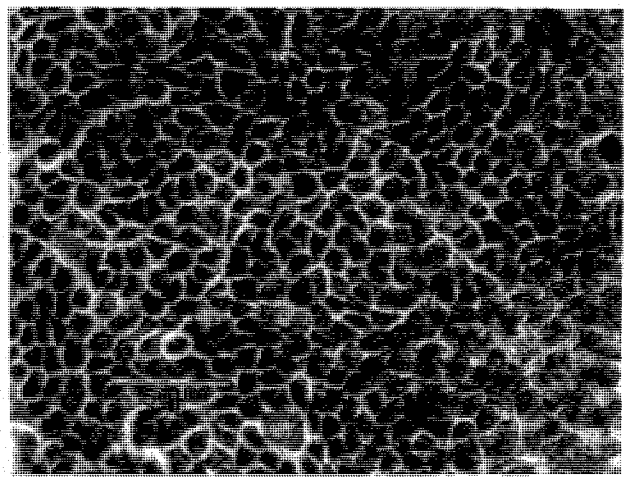

Fig. 2. Phase-contrast micrograph of a MDCK cell (ATCC strain) monolayer grown over a Transwell system for 6 days. The sample was fixed with glutaraldehyde, stained with hematoxylin/eosin, and clarified in chloroform. Bar indicates 50 μm.

mulated percentage mass transported from the total starting amount is reported as a function of time. The percentage mass transported of the fluid-phase markers of β-emitter was calculated from the dpm measured on a Beckman Model SL-5801 liquid scintillation counter. Optical quenching due to the presence of LY appeared to be minimum; the percentage efficiency of the dpm reading was of the order of 45% for ^{3}H- and 75% for ^{14}C-radionuclides.

RESULTS AND DISCUSSION

As shown in Fig. 2, MDCK cells formed a confluent monolayer on the polycarbonate membrane in the Transwell system even without any matrix treatment. Staining with hematoxylin/eosin generally worked well for a light microscopic examination, displaying blue nuclear structures and pinkish cytoplasmic components. The cuboidal cells with a well-defined junctional space were, on the average, 12–15 μm in size. That the cells formed a monolayer is perhaps better illustrated in a transmission (TEM) or a scanning electron micrograph (SEM). As shown in Fig. 3, the cell monolayer was approximately 6–8 μm thick. In addition to intercellular lateral space, a close examination revealed a few tight junctions (arrowheads in Fig. 3). The polycarbonate membrane was about 14 μm thick and showed well-defined 3-μm pores. To reveal the underneath polycarbonate membrane in the SEM, the cell monolayer sample shown in Fig. 4 was partially lifted by a brief treatment (<5 min) with an EDTA/trypsin solution. Generally, a similar morphology was observed for the strain I cell monolayers, however, the average cell dimension appeared somewhat smaller than that of the ATCC strain.

The cell growth for both strains showed a typical sigmoidal profile (data not shown). The lag phase depended upon the seeding concentration. The growth phase showed a doubling time of the order of 21 hr for both strains, which agrees well with the 22 hr reported (14). Similarly, the cell density observed at confluence, about 3.7×10^5 cells/cm^2, also agrees well with the literature value of 4.0×10^5 cells/cm^2 (14). Even beyond that point, the cells were found to grow continuously along the side of the Transwell. This was easily detected by hematoxylin/eosin stain. Also, a large deviation in cell recovery was observed after day 7.

Concurrent with the cell growth was the increase in TEER. As shown in Fig. 5, on day 4, two different strains began to show a dramatic difference in TEER. On day 5, the value appeared to have reached a constant value, in unison with the cell growth. As expected, the strain I cells resulted in much higher TEER values, frequently in the range of 1500 $\Omega \cdot cm^2$. However, greater variations were observed with the strain I cell layers. The exact cause for this observation is not known. It was noticed that the pH of the medium for strain I became acidic more rapidly than that for the ATCC strain during the incubation, implying that medium change once a day may not be sufficiently frequent for the growth of strain I in the Transwell system used. In some instances, TEER was measured on the same monolayers over a few-day period, before and after transport studies (see below). For instance, of 41 measurements on day 5 for the ATCC strain, 12 pairs of TEER values are those measured before and after transport studies and 6 monolayers were also used on day 4. Since TEER was not measured in an aseptic environment, occasionally bacterial growth was observed. Data from these samples were discarded. In one series of

Fig. 3. Transmission electron micrograph of an MDCK cell (ATCC strain) monolayer. Two nuclei (N) are shown along with tight junctions (arrowheads) and microvilli (MV) on the apical membrane. Some of the 3-μm pores in the polycarbonate membrane (asterisks) appeared as doublets. Flat cell monolayers were also commonly observed. Bar indicates 5 μm.

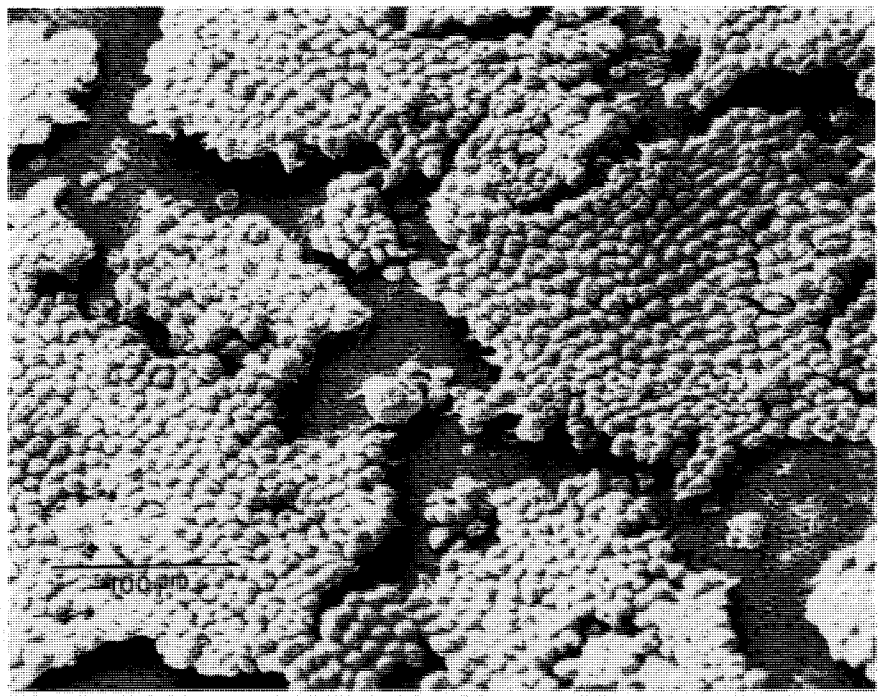

Fig. 4. Scanning electron micrograph of a partially lifted MDCK cell (ATCC strain) monolayer. The underlying polycarbonate membrane shows 3-μm pores. The sample was obtained by a brief treatment of the cell monolayer with an EDTA/trypsin solution and prepared for the SEM following a normal procedure. Bar indicates 100 μm.

experiments, both strains were used in developing mixed cell monolayers. On day 5, the average TEER of these samples was, as expected, identical to that observed with the ATCC cell monolayers within experimental error. Finally, TEER values reported here appear to be significantly higher for the ATCC strain but lower for the strain I than those reported in the literature (5–7).

In the present study, the transport of four different fluid-phase markers across monolayers of two different MDCK cell strains was monitored at 37 and 4°C in two different directions. A test solution contained either [^{14}C]sucrose, LY, and [^{3}H]inulin or [^{14}C]sucrose, LY and [^{3}H]dextran. At 37°C, all possible combinations of these variables were studied. At 4°C, however, only limited amounts of data were collected for protocol 2, in which the transport occurred in the apical-to-basolateral direction. Sucrose transport in the basolateral-to-apical direction (i.e., protocol 1) is exemplified in Fig. 6 for two cell monolayers with dramatically different permeabilities. Transport rates reported in the present study are all determined from the data obtained at $t > 60$ min, where a steady-state rate was observed. Typical data are summarized in Table I for one series of experiments, along with TEER measured before and after the transport experiments. The rates are listed in the order of "tightness" of the cell monolayer toward the sucrose molecule.

As illustrated in Table I, a large variation was observed between the cell monolayer preparations. No rigorous attempt was made to reproduce monolayers as tight as possible or to discard leaky preparations prior to the tranport studies. In some studies (9), rejection as high as 80% was reported in preparing "leakage-free" MDCK cell monolayers. The magnitude of the variation is such that it is of little significance to assign an absolute value for the transport of a specific probe molecule. Instead, we used the transport rate of [^{14}C]sucrose as a reference. The rate ratios are listed in Table II for all data obtained at 37°C. The overall average

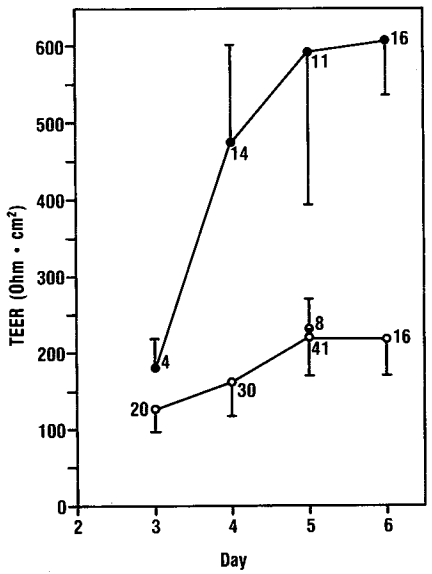

Fig. 5. TEER of MDCK cell monolayers as a function of incubation time; strain from ATCC (open symbols) and strain I (filled symbols). Vertical bars indicate one standard deviation and numbers represent the number of determinations. The half-filled circle on day 5 represents TEER of cell monolayers containing both strains.

ratio for the transport of sucrose (MW 342), LY (457), inulin (5000–5500), and dextran (50,000–125,000) was 1.00:0.80:0.67:0.15. At 4°C, the only statistically significant data collected are the rate ratios of LY to sucrose for the protocol 2 study. They are 0.79 (N = 6) and 0.85 (N = 4) for the ATCC and strain I cell layers, respectively.

Highly permeable cell monolayers such as Sample No. 7.20.2 in Table I must represent leaky preparations. On the other hand, there is no direct experimental evidence that the transport across a preparation such as Sample No. 8.8.2 represents exclusively a transcellular vesicular transport. In fact, it has been proposed that the monolayer of MDCK cells has a paracellular permeation route at a TEER in the range of 200 Ω · cm^2 and that the paracellular path has large variations along the perimeter of a given cell (15). It appears to be a difficult task to assess quantitatively the contribution of each of the paracellular and the transcellular transport mechanisms to the observed transport. It is primarily because there is no established procedure to turn off one mechanism without affecting the other. The use of selective poisoning or low temperatures to abolish the energy-requiring transcytosis, for example, entails the assumption that the integrity of tight junctions remains intact for a certain period of time. The duration of a transport experiment should be long enough for a measurable amount of marker molecules to pass through the cell layer. In the absence of paracellular leakage, as reported for the normal intestinal epithelium (16) or for the most rigorously selected strain I MDCK cell monolayers (9), the transport observed would be best analyzed in terms of cellular uptake and efflux. Here, the vesicle shuttling was only recently subjected to compartmental analysis (2,12,17) and is far from being firmly established. In short, the idea of separating the paracellular shunt pathway from transcellular transport remains convenient in concept (1) but elusive in practice.

Table I. Transepithelial Electrical Resistance (TEER) and Apical-to-Basolateral Transport Rates of Sucrose, Lucifer Yellow CH (LY), Inulin, and Dextran Across 4.71-cm^2 Monolayers of the ATCC Strain of MDCK Epithelial Cells at 37°C

Sample No.[a]	TEER (Ω-cm^2)[b]	Steady-state rate (%-hr^{-1})			
		Sucrose	LY	Inulin	Dextran
8.8.2	232–265	0.044	0.033	—	0.010
8.11.1	180–288	0.049	0.035	0.044	—
8.7.1	208–283	0.091	0.071	0.073	—
7.17.1	184–254	0.166	0.121	(0.190)[c]	—
7.19.2	198–240	0.186	0.138	—	0.023
3.3.2	183–201	0.325	0.310	0.270	—
7.18.1	179–268	0.357	0.277	(0.240)[c]	—
8.10.2	265–288	0.409	0.422	—	0.243
3.8.3	211–225	0.588	—	—	0.080
3.2.1	230–244	0.688	0.490	—	—
3.4.2	178–183	0.689	0.670	0.460	—
3.5.3	178–211	0.723	0.570	—	0.105
3.1.1	211–98	0.768	0.589	—	—
7.20.2	141–184	1.50	1.25	—	0.240

[a] Samples are identified as Expt. No–Well No.–Soln. No.
[b] The two numbers correspond to TEER values measured immediately before and after the transport experiments.
[c] An inulin solution, which had been stored at 4°C for 30 days after purification was used. These data were not used in Table II.

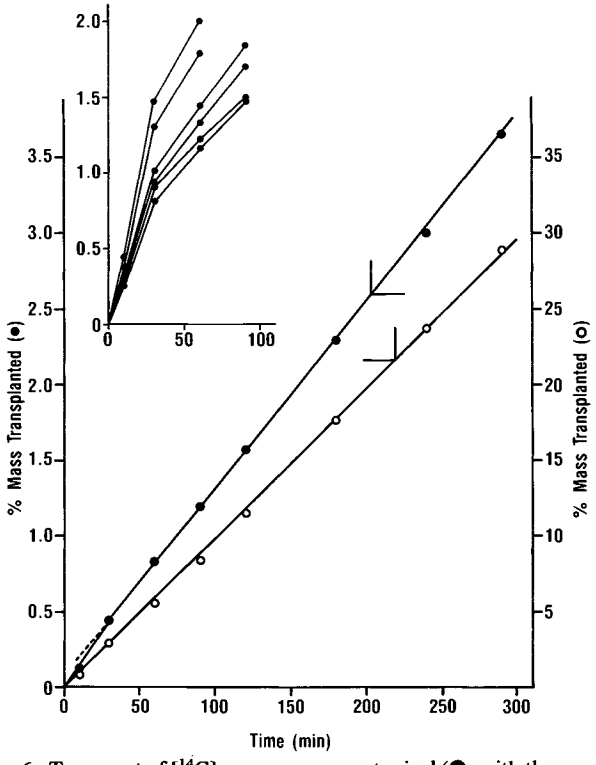

Fig. 6. Transport of [^{14}C]sucrose across a typical (●, with the y axis on the left) and an extremely leaky (○, with the y axis on the right) strain I MDCK cell monolayer over a 290-min period at 37°C. In both cases, the transport took place from basolateral to apical direction (protocol 1). Inset displays early time points for LY transport across six different ATCC strain cell monolayers in the apical-to-basolateral direction (protocol 2). The concentrations of sucrose and LY were 2.17 μg/ml (no cold sucrose added) and 0.457 mg/ml, respectively.

In contrast to the large variations in the transport rates observed, the rate ratio of 1.00:0.80:0.67:0.15 for sucrose, LY, inulin, and dextran appears to remain constant: independent, within experimental errors, of temperature, cell strain used, direction of solute migration, and TEER. This observation supports, if not proves, a common transport mechanism. The slowest initial transport rate of [^{3}H]dextran observed in our study from the basolateral side of a strain I MDCK cell monolayer was approximately 9.0 × 10^{-6} nl · cell^{-1} · min^{-1} (raw data not shown). Had this been exclusively via transcellular transport, the uptake rate would have been of the order of 7 × 10^{-5} nl · cell^{-1} · min^{-1}. This estimation is based on the uptake-to-transcytosis ratio reported by von Bondsdorff *et al.* for the same strain of MDCK cells (9). Our calculated uptake rate is still approximately 300-fold greater than the value von Bonsdorff *et al.* reported. This analysis is thus supportive of the paracellular transport throughout the present study. The transport rates listed in Table I as well as those measured in other experiments were insensitive to the observed TEER values. If the latter is related to the sealing capacity of the intercellular junction and the resistance of the lateral space (15), one would expect some correlation between TEER and paracel-

Table II. Ratio of Steady-State Transport Rates of Lucifer Yellow CH (LY), Inulin, and Dextran to That of Sucrose at 37°C

	Protocol 1. Transport in basolateral-to-apical direction						Protocol 2. Transport in apical-to-basolateral direction					
	LY		Inulin		Dextran		LY		Inulin		Dextran	
	ATCC	Strain I	ATCC	Strain I	ATCC	Strain I	ATCC	Strain I	ATCC	Strain I	ATCC	Strain I
N	9	8	4	4	4	4	14	16	4	7	5	8
$\bar{X}$	0.829	0.767	0.580	0.628	0.136	0.134	0.807	0.807	0.795	0.671	0.156	0.192
Min	0.665	0.638	0.522	0.581	0.121	0.099	0.704	0.700	0.668	0.526	0.124	0.105
Max	0.989	0.947	0.686	0.681	0.158	0.206	1.03	1.11	0.881	0.814	0.216	0.396
SD	0.088	0.088	0.073	0.044	0.017	0.050	0.103	0.109	0.091	0.098	0.036	0.103

lular permeability. Electrical conductance, or resistance in reciprocal, in solutions is due to migration of ions. As such, it may not reflect the transport rate of neutral molecules. Exactly what relationship exists between TEER and paracellular permeability of nonelectrolytes remains to be investigated further.

The rate ratio observed appears to reflect not only the size but also the charge of the probe molecules. The former dictates the diffusion coefficient, whereas the latter would affect the transport rate through electrostatic interactions with the surrounding environment. For instance, electrostatic repulsion between negatively charged tight junctions (18,19) and LY molecules may account for the slower transport rate than sucrose. Although the [^{3}H]inulin used was purified by size-exclusion chromatography prior to transport experiments, potential ^{3}H exchange with the medium (9) still exists. This would prevent any firm comparison with other transport rates.

In summary, the present study characterized MDCK epithelial cell monolayers grown over a polycarbonate membrane. The system can be easily obtained for systematic studies on the structure–cellular transport relationship. One important caveat is the difficulty with which the overall transport can be separated into each of the paracellular and the transcellular transport mechanisms.

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