

Transepithelial ultrafiltration and fractal power diffusion of D-glucose in the perfused rat intestine

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Abstract

Despite an enormous body of research investigating the mass transfer of D-glucose through biological membranes, carrier-mediated and first-order models have remained the prevalent models describing glucose's quantitative behavior even though they have proven to be inadequate over extended concentration ranges. Recent evidence from GLUT2 knockout studies further questions our understanding of molecular models, especially those employing Michaelis–Menten (MM)-type kinetic models. In this report, evidence is provided that D-glucose is absorbed by rat intestinal epithelium by a combination of convective ultrafiltration and nonlinear diffusion. The diffusive component of mass transfer is described by a concentration-dependent permeability coefficient, modeled as a fractal power function. Glucose and sodium chloride-dependent-induced aqueous convection currents are the result of prevailing oncotic and osmotic pressure effects, and a direct effect of glucose and sodium chloride on intestinal epithelium resulting in enhanced glucose, sodium ion, and water mobility. The fractal power model of glucose diffusion was superior to the conventional MM description. A convection–diffusion model of mass transfer adequately characterized glucose mass transfer over a 105-fold glucose concentration range in the presence and absence of sodium ion.

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1. Introduction

Despite more than 50 years of research investigating glucose biological membrane translocation, the mechanisms of glucose transport remain controversial, and an adequate comprehensive quantitative model describing glucose mass transfer has proven to be elusive [1,2]. As early as 1952, Widdas [3] demonstrated that glucose mass transfer was nonlinear in human erythrocytes and sheep placenta. He introduced the notion that “passive” glucose transport could be quantitatively characterized by simple Michaelis–Menten (MM)-type kinetics, which later lead to the concept that glucose membrane transport was carrier mediated. This model was modified in 1975 to include both MM transport kinetics and a true first-order diffusive component [4] to account for the observation that a saturation asymptote,

implicit in MM kinetics, could not be experimentally detected even up to glucose concentrations of several hundred millimolars.

Models incorporating MM (or closely related modifications) and/or first-order components of glucose mass transfer remain as the staple today for the quantitative description of glucose mass transfer for whole tissue, cells, and vesicles [2,5–9] although they have proven to be inadequate over an extended range of glucose concentrations as well as in the quantitative description of other related sugars [2].

In an attempt to account for deficiencies in these models, Pappenheimer and Reis [10] introduced a model in 1987 for paracellular mass transfer by convective streaming (solvent drag). This model was based on nonequilibrium thermodynamic concepts which maintain that solute transport through a semipermeable membrane will necessarily lead to both ordinary diffusion and coupled ultrafiltration [11]. The driving force associated with ultrafiltration is a dynamically coupled pressure gradient caused by the relative selectivity of the membrane toward solute compared to solvent, usually water. It had long been established that isolated small

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intestine was able to absorb fluid even in the apparent absence of any external driving force [12,13]. Subsequent investigation demonstrated that water absorption was enhanced in the presence of D-glucose and sodium chloride [13,14]. Pappenheimer and Reis [10] were able to show that glucose absorption was correlated to convective streaming.

Although convective mass transfer was not considered in earlier studies, MM kinetic models have persisted in the molecular quantitative description of glucose mass transfer. The persistence of MM kinetics as quantitative models may be attributable in large measure to the putative inhibitory effects of phloridzin and phloretin on glucose transport at the SGLT1 and GLUT2 domains, respectively. The findings of two recent studies, however, suggest alternative mechanisms to those usually considered.

When convective mass transfer was considered in later studies [15–17], the nonconvective component of mass transfer was modeled by MM kinetics and the first-order component ignored. Thus, values of K_m are reported for SGLT1-mediated transport even though a 3.6-fold increase in glucose absorption rate is observed between 50 and 150 mM glucose [18]. In the presence of the β -glucosides, phloridzin and phloretin, reported K_m values for glucose mass transfer are dramatically altered [4,7]. These latter effects appear consistent with MM model predictions of competitive inhibition of a molecular intermediate, even though asymptotic glucose mass transfer is not observed. Additionally, the SGLT1 and GLUT2 domains currently are thought to function as highly selective glucose transporter intermediaries.

Recently, gum arabic, a mixture of monosaccharides, was shown to cause partial reversal of the inhibitory effect of phloridzin and complete reversal of the inhibitory effect of phloretin toward D-glucose mass transfer in perfused rat intestine [19]. This study does not support the role of phloridzin and phloretin as competitive inhibitors, since to displace phloridzin and phloretin from SGLT1 and GLUT2 domains, gum arabic must also act as an efficient competitive inhibitor, and not as a promoter of glucose mass transfer.

Even more convincing is a study comparing the absorption of glucose from isolated perfused intestine of wild-type mice and GLUT2 knockout mice in which no difference in glucose mass transfer was found between the two types of intestinal epithelia [20]. These results indicate that a transport intermediary is not responsible for glucose absorption, at least with regards to the GLUT2 domain.

Paradoxically, Kellett [1] and Kellett and Helliwell [7] report that GLUT2-mediated mass transfer is the principal route for glucose absorption and that excised jejunum or other *in vitro* preparations result in inactivation of protein kinase C β II and loss of GLUT2 from brush-border membrane, thereby rendering results from the use of such preparations irrelevant with regards to *in vivo* absorption. The conclusions from this study, however, are mechanistically inconsistent with the results in GLUT2 knockout mice,

suggesting that *in vitro* experiments may not provide adequate evidence to support current mechanistic models of glucose mass transfer.

These studies suggest that problems inherent in current quantitative models of glucose mass transfer may be related to a lack of understanding of the nonconvective component of glucose absorption and inadequate quantitative characterization of this component, which is complicated by the confounding convective streaming effect.

In the present study, we report that an adequate comprehensive quantitative model describing glucose mass transfer can be achieved by considering convective mass transfer and a nonlinear diffusive component modeled as a fractal power function. This model was evaluated with glucose concentrations extending over a 105-fold concentration range in the presence and absence of sodium ions. We also evaluated the relative effects of sodium chloride and glucose concentrations on hydraulic conductivity in the perfused rat intestine.

2. Materials and supplies

D-(+)-Glucose, D-mannitol, and sodium chloride were obtained from Sigma (St. Louis, MO). High-purity water was obtained from Burdick & Jackson (Muskegon, MI). D- 14 C(U)glucose was obtained from Du Pont NEN (Boston, MA). Acepromazine and ketamine were supplied by A.J. Buck (Owings Mills, MD). Cannulae were made from polyethylene PE 280 tubing supplied by Clay Adams (Parsippany, NJ).

Instrumentation included a Packard Tri-Carb Model 1500 liquid scintillation analyzer (Meriden, CT), Harvard Model 950 infusion pump (South Natick, MA), Accumet Model 825 MP pH meter and Orion sodium-ion selective electrode supplied by Fisher Scientific (Fair Lawn, NJ).

Male Sprague–Dawley rats weighing 300–350 g were obtained from Charles River Breeding Laboratory (Kingston, NJ).

3. Methods

3.1. Perfusion solutions

For hydraulic conductivity and glucose convection–diffusion experiments, perfusate solutions were prepared to contain various concentrations of D-glucose ranging from 2.5 to 70 mg/ml, and 2.5 or 7.5 mg/ml sodium chloride in ultrapurified water. Each solution additionally contained a trace amount of D- 14 Cglucose resulting in a final radioactive concentration of ~ 4.5 nCi/ml (0.2 ng/ml). The effect of sodium chloride on hydraulic conductivity at an isometric glucose concentration was evaluated with perfusates containing various concentrations of sodium chloride ranging from 0 to 9 mg/ml, and 10 mg/ml D-glucose.

For glucose diffusion experiments, perfusates were prepared to contain various concentrations of D-glucose ranging from 0.5 to 50 mg/ml, either 0 or 2.5 mg/ml sodium chloride in ultrapurified water, and 4.5 nCi/ml of D-[^{14}C]glucose. In addition, 19, 23, 21, 22, 17 and 11 mg/ml of D-mannitol were added as an osmotic rectifying agent to perfusates containing 2.5 mg/ml of sodium chloride and 5, 7.5, 10, 15, 20, and 30 mg/ml of D-glucose, respectively. The concentration of mannitol required to minimize aqueous convective streaming was determined from preliminary experiments. All other solutions did not contain any mannitol.

3.2. Intestinal perfusion technique

Radial epithelial glucose mass transfer and aqueous convective streaming were evaluated using the in situ single-pass intestinal perfusion method. All animal studies adhered to the “Principles of Laboratory Animal Care” (NIH publication #85-23, revised 1985). The procedure employed is as follows. Following an overnight fast from rat chow, anesthesia was induced approximately one-half hour before surgery with 1 mg/kg acepromazine and 90 mg/kg ketamine administered intramuscularly. Additional booster doses were given as required to maintain anesthesia during the experiment at one-fourth the initial dose. The rats were placed under a surgical lamp to maintain body temperature. The small intestine was exposed by a midline abdominal incision and a 5-cm inlet cannula inserted through a small incision in the intestine in the vicinity of the ligament of Treitz. A similar outlet cannula was inserted approximately 10–15 cm distally in situ (corresponding to ~ 20–25 cm when exteriorized and extended). Both cannulae were secured by ligatures. The inlet cannula was connected to an infusion pump and the appropriate glucose solution perfused at a calibrated rate of 0.51 ml/min. Inlet solutions were maintained at ~ 37 °C by warming the perfusate to 39 °C. The perfused intestinal segment was repositioned in the abdominal cavity such that unimpeded axial flow was maintained, and the abdominal incision closed with wound clips. The intestinal preparation was equilibrated with the appropriate perfusate for 60 min. Six (6) consecutive 10-min outlet samples of perfusate were then collected. At the conclusion of each experiment, the perfused intestinal segment was excised and blotter dried. The segment was then weighed. Each distinct experiment corresponding to each perfused solution was replicated in 3–10 rats for hydraulic conductivity experiments and 3–6 rats for convective–diffusive experiments.

3.3. Analytical and computational methods

D-[^{14}C]glucose was quantified using liquid scintillation analysis. 0.5-ml aliquots of inlet and outlet perfusate samples were mixed with 10 ml of liquid scintillation cocktail and counted. Counts per minute (cpm) were converted to disintegrations (dpm) by a quench curve. Sodium ion

concentrations were determined by sodium-ion selective potentiometry.

Outlet concentrations from the six post-equilibrated interval collections were analyzed for time-related trends. In the absence of any systematic trends, steady state was assumed. The concentration of D-[^{14}C]glucose or sodium ion from the six 10-min interval samples were then averaged and considered the final steady-state outlet concentration.

Outlet volumetric flow rates were determined gravimetrically for each 10-min interval. Similarly, in the absence of any systematic time-related trends, steady state was assumed and the values averaged.

The steady-state transepithelial aqueous volume flux (J_v) was calculated by:

$$J_v \text{ (ml/min/g)} = [Q_{\text{in}} - Q_{\text{out}}] / \text{weight of segment} \quad (1)$$

where Q_{in} and Q_{out} are the inlet and outlet volumetric flow rates, respectively. Net mucosal-to-serosal aqueous absorption is reported as positive values and net serosal-to-mucosal secretion as negative values.

The steady-state transepithelial glucose flux (J_{gluc}) was calculated from the D-[^{14}C]glucose radioactive concentration as follows:

$$J_{\text{gluc}} \text{ (mg/min/g)} = (C_{\text{in}}Q_{\text{in}} - C_{\text{out}}Q_{\text{out}}) / \text{weight of segment} \quad (2)$$

where C_{in} and C_{out} are the respective inlet and outlet glucose concentrations, and

$$C_{\text{out}} = C_{\text{in}}[\text{dpm}_{\text{out}} - \text{dpm}^\circ] / [\text{dpm}_{\text{in}} - \text{dpm}^\circ] \quad (3)$$

where dpm° is the background response of a scintillation blank. Sodium ion flux (J_{Na^+}) was similarly calculated from Eq. (2).

The perfused segment glucose concentration corresponding to the transepithelial flux was considered the geometric average of axial concentration, $[C]$, such that,

$$[C] = (C_{\text{in}} - C_{\text{out}}) / \ln(C_{\text{in}}/C_{\text{out}}) \quad (4)$$

4. Data analysis

Diffusive mass transfer of glucose was analyzed by the following nonlinear model,

$$J_{\text{gluc}} = P_e(C)[C] \quad (5)$$

where the concentration-dependent permeability coefficient, $P_e(C)$, was empirically evaluated. The functional dependency of $P_e(C)$ was best described by fractal power function such that,

$$P_e(C) = P^\circ [C]^{-\beta} \quad (6)$$

where, $\beta \in \Re [0,1]$.

Convective–diffusive mass transfer was analyzed by a linear combination model such that,

$$J_{\text{gluc}} = \alpha J_v[C] + P_e(C)[C] \quad (7)$$

where the functional dependency of $P_e(C)$ on glucose concentration was equivalent to that of the nonconvective model. α is the coefficient of ultrafiltration and generally regarded as equal to $1 - \sigma$, where σ is defined as the reflection coefficient [11].

Substitution of Eq. (6) into Eqs. (5) and (7) yields the final models,

$$J_{\text{gluc}} = P^\circ [C]^{1-\beta} \quad (8)$$

$$J_{\text{gluc}} = \alpha J_v[C] + P^\circ [C]^{1-\beta} \quad (9)$$

for diffusion and convection–diffusion, respectively.

Correlation of data with the prescribed models was analyzed by nonlinear and linear multivariate least-squares regression techniques. Comparative evaluation of different models used the Akaike information criterion (AIC). Regression parameter comparisons and other comparative analyses used standard statistical methods.

5. Results

5.1. Inductive transport and hydraulic conductivity

The transepithelial aqueous flux showed a bivariate dependency on glucose and sodium chloride concentration (Fig. 1). Perfusates initially containing no sodium chloride generated a mean (\pm S.E.) maximum aqueous flux of 27 $\mu\text{L}/\text{min}/\text{g}$

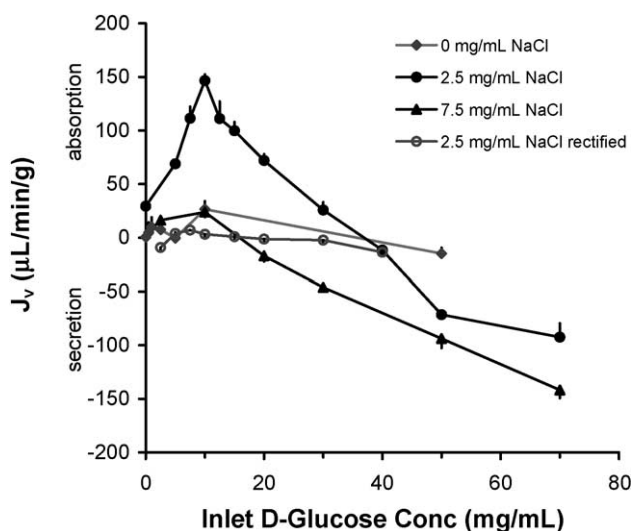


Fig. 1. Glucose-mediated transepithelial aqueous flow rate and its mutual dependence on sodium concentration. Each value is the mean (\pm S.E.) of 3–10 determinations. Inlet NaCl concentrations of 0, 2.5 and 7.5 mg/ml corresponded with mean $[\text{Na}^+]$ of 0.3, 1.2 and 2.9 mg/ml at steady state.

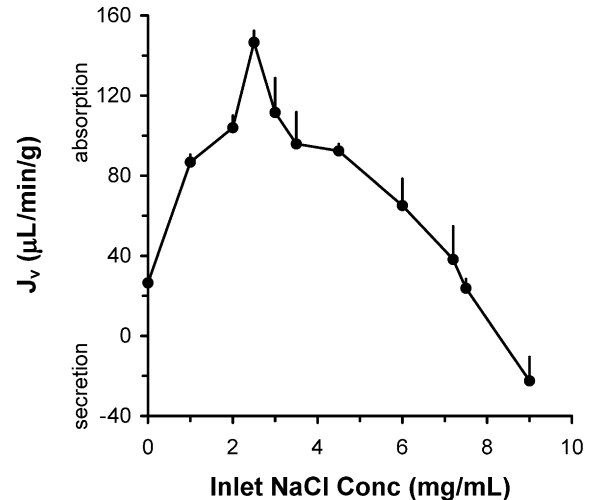


Fig. 2. Dependence of the transepithelial aqueous flow rate on NaCl concentration at an inlet D-glucose concentration of 10 mg/ml. Each value is the mean (\pm S.E.) of 3–10 determinations.

min/g (± 7.6 , $n=8$) at 10 mg/ml glucose. This effect was significantly amplified by the addition of 2.5 mg/ml sodium chloride to the perfusate. The dependency of the inductive effect on sodium chloride (Fig. 2) showed a unimodal global maximum J_v of 147 $\mu\text{L}/\text{min}/\text{g}$ (± 5.8 , $n=10$) at 2.5 mg/ml sodium chloride and 10 mg/ml glucose. Maximum relative glucose-dependent hydraulic conductivities were observed at 10 mg/ml glucose for all sodium chloride concentrations tested. Flux declined monotonically at glucose concentrations greater than 10 mg/ml at all tested sodium chloride concentrations. The aqueous flux resulting from the oncotic pressure in the absence of both glucose and sodium chloride in the perfusate was 0.5 $\mu\text{L}/\text{min}/\text{g}$ (± 9.92 , $n=7$). At 2.5 mg/ml sodium chloride, J_v values were 29 ± 11.5 ($n=3$), 69 ± 3.0 (4), 111 ± 10.9 (4), 147 ± 5.8 (10), 111 ± 16.0 (4), 100 ± 8.1 (3), 72 ± 6.0 (4), 26 ± 7.3 (3), -12 ± 3.1 (6), -71 ± 3.5 (3), and -92 ± 12.9 (4) $\mu\text{L}/\text{min}/\text{g}$, corresponding to initial glucose concentrations of 0, 5, 7.5, 10, 12.5, 15, 20, 30, 40, 50, and 70 mg/ml, respectively. At 10 mg/ml glucose, J_v values were 27 ± 7.6 (8), 87 ± 3.8 (3), 104 ± 6.1 (5), 147 ± 5.8 (10), 112 ± 17.2 (7), 96 ± 15.9 (5), 92 ± 3.5 (5), 65 ± 13.4 (3), 38 ± 16.6 (4), 24 ± 4.7 (5), and -23 ± 12.0 (4) $\mu\text{L}/\text{min}/\text{g}$, corresponding to initial sodium chloride concentrations of 0, 1, 2, 2.5, 3, 3.5, 4.5, 6, 7.2, 7.5, and 9 mg/ml, respectively. The global maximum inductive effect at 10 mg/ml glucose and 2.5 mg/ml sodium chloride corresponded to average steady-state axial concentrations, $[C]$, of 9.1 mg/ml glucose and 1.34 mg/ml sodium ion in the perfused intestinal segment. These results show that when compared to the oncotic aqueous flux, both sodium chloride and glucose can act independently as aqueous flux inducers. However, the mutual effect of the combination of glucose and sodium chloride results in an enhanced J_v of 147 $\mu\text{L}/\text{min}/\text{g}$, or greater than 2.5 times the sum of the independent contributions, 27 and 29 $\mu\text{L}/\text{min}/\text{g}$, respectively, at the global maximum.

The inductive effect associated with perfusate concentrations of 2.5 mg/ml sodium chloride and various glucose concentrations ranging from 5 to 30 mg/ml was virtually abolished with the addition of mannitol concentrations ranging from 11 to 23 mg/ml to the perfusate. J_v values were 3.9 ± 1.5 (5), 7.1 ± 0.2 (3), 3.2 ± 4.2 (4), 0.9 ± 4.1 (3), -1.3 ± 2.1 (6), and -2.3 ± 1.9 (6) $\mu\text{l}/\text{min}/\text{g}$ with corresponding mannitol concentrations of 19, 23, 21, 22, 17, and 11 mg/ml and glucose concentrations of 5, 7.5, 10, 15, 20, and 30 mg/ml, respectively. These values represent reductions in free convection (no mannitol) as measured by the difference in J_v with osmotic rectification and the nominal free convection values relative to the free convection values of 94.3%, 93.6%, 97.9%, 99.1%, 101.8% and 108.9%.

To assess the specificity of the inductive effect for water, glucose and sodium ion fluxes were evaluated with isodynamic forces and a variable auxiliary chemical potential (Table 1). Sodium ion serosal-to-mucosal flux was amplified 57-fold with the addition of 50 mg/ml glucose to the intestine mucosal surface even though the luminal sodium chloride chemical potentials were isometric and nearly isobaric conditions prevailed as determined by cohort matched aqueous fluxes. Similarly, glucose mucosal-to-serosal flux was amplified 2.8-fold with the addition of 7.5 mg/ml sodium chloride to the mucosal surface with isometric glucose chemical potentials and isobaric convection. In both instances, the effect of the auxiliary chemical potential was highly significant ($P < 0.001$). These findings suggest that the combination of mucosal glucose and sodium chloride directly affects intestinal epithelial properties resulting in enhanced molecular mobility. Furthermore, the inductive effect appears to be relatively nonspecific. The 293-fold increase in hydraulic conductivity relative to oncotic hydraulic conductivity and associated with the mutual combination of glucose and sodium chloride therefore appears to be the result of enhanced water mobility and not related to any force induction.

5.2. Glucose diffusion

Ideally, glucose ultrafiltration must be eliminated to isolate the nonconvective (diffusive) component of glucose

mass transfer. Since glucose also affects hydraulic conductivity and induces convective streaming, it was apparent that experimental designs would require reversal of the inductive effect while retaining the flexibility to study the diffusive component within a variable glucose concentration range. In addition, since sodium chloride was also found to affect hydraulic conductivity, two sets of experiments were designed with differing conditions. The objective of the first set of experiments was to provide a baseline characterization of glucose binary diffusivity utilizing a simple perfusate of only glucose in water since diffusion in multi-component systems can give rise to the so-called “cross-term” diffusivities associated with pairwise interactions of glucose and each additional solute present in the perfusate [11]. Eliminating sodium chloride was especially important in view of the effect it demonstrated in altering glucose mobility. The above studies concerning hydraulic conductivity also showed that convective streaming would be minimal with a perfusate glucose concentration range of 0.5–50 mg/ml.

The objective of the second set of experiments was to evaluate the effect of sodium chloride on glucose binary diffusivity. To accomplish this goal, experimental conditions required that convective streaming be nullified while still retaining the intrinsic effect of sodium chloride on glucose mobility in the intestinal epithelia. This was accomplished by including various concentrations of mannitol in perfusates as an osmotic rectification agent to produce conditions isobaric with the first set of experiments.

In the absence of initial sodium chloride in the perfusate, mean (\pm S.E.) steady-state glucose flux values, J_{gluc} , were 0.0421 ± 0.0081 ($n=4$), 0.0715 ± 0.0163 (5), 0.128 ± 0.011 (4), 0.235 ± 0.045 (3), 0.461 ± 0.117 (3), and 1.69 ± 0.27 (3) mg/min/g, corresponding to average axial glucose concentrations $[C]$ of 0.449 ± 0.009 , 0.910 ± 0.013 , 2.32 ± 0.02 , 4.73 ± 0.04 , 9.61 ± 0.06 , and 47.3 ± 0.5 mg/ml. The average axial Na^+ concentration resulting from epithelial secretion was 0.278 mg/ml. Aqueous flux values ranging from -15 to 13 $\mu\text{l}/\text{min}/\text{g}$ were observed in these experiments with the minimum value occurring at 50 mg/ml initial glucose and maximum value occurring at 10 mg/ml.

In the presence of an initial sodium chloride concentration of 2.5 mg/ml and isobaric mannitol concentrations,

Table 1
Effect of D-glucose and sodium chloride perfusate concentration on sodium ion and glucose flux

D-Glucose concentration (mg/ml)	Sodium chloride concentration (mg/ml)	J_v ($\mu\text{l}/\text{min}/\text{g}$)	J_{Na^+} ($\mu\text{g}/\text{min}/\text{g}$)	J_{gluc} ($\mu\text{g}/\text{min}/\text{g}$)
50	0	-15 ± 9.4 ; 3 ^a	-272 ± 48.6 ; 3	
0	0	-9.5 ± 7.68 ; 5	-4.8 ± 3.52 ; 5	
	<i>P</i> -value	0.413	<0.001	
10	0	13 ± 12.0 ; 3		461 ± 202.7 ; 3
10	7.5	24 ± 10.3 ; 5		1285 ± 56.2 ; 5
	<i>P</i> -value	0.222		<0.001

^a Mean \pm S.D.; n .

the mean (\pm S.E.) steady-state glucose flux values were 0.640 ± 0.042 ($n=5$), 0.821 ± 0.028 (5), 0.980 ± 0.136 (3), 1.30 ± 0.15 (4), 1.37 ± 0.15 (3), 1.66 ± 0.10 (4), 1.91 ± 0.10 (6) and 2.48 ± 0.43 (5) mg/min/g, corresponding to average axial glucose concentrations $[C]$ of 1.70 ± 0.03 , 3.96 ± 0.04 , 6.22 ± 0.20 , 8.28 ± 0.13 , 13.3 ± 0.28 , 17.8 ± 0.12 , 27.7 ± 0.20 , and 36.8 ± 0.32 mg/ml. The average axial Na^+ concentration was 1.15 mg/ml. Aqueous flux values ranging from -12 to 7.1 $\mu\text{l}/\text{min}/\text{g}$ were observed in these experiments with the minimum value occurring at 40 mg/ml glucose and maximum value occurring at 7.5 mg/ml.

Regression analysis indicated that the transport kinetics of glucose (Fig. 3) were best characterized by a two-parameter glucose concentration-dependent permeability model whereby the permeability function was described by a fractal power function in accord with Eq. (8). The mathematical formalism of this relationship was invariate with differing membrane transport properties associated with the different sodium chloride concentrations with R^2 values exceeding 0.97 in both instances. Parametric values, however, showed a marked dependence on sodium chloride concentration with a 6.5-fold increase in P° and 44% reduction in the exponential power, $1 - \beta$ (Table 3). These differences were associated with about a 4-fold increase in sodium ion concentration in the perfused intestinal segment. Parameter values corresponding to the different conditions were statistically distinguishable as demonstrated by non-overlapping 90% confidence intervals.

For comparison, the fractal power model was statistically compared to a simple MM model with a two-parameter family. Comparison of a third model was attempted combining the MM model and a first-order diffusion component, but analysis was indeterminate since one or more parameter

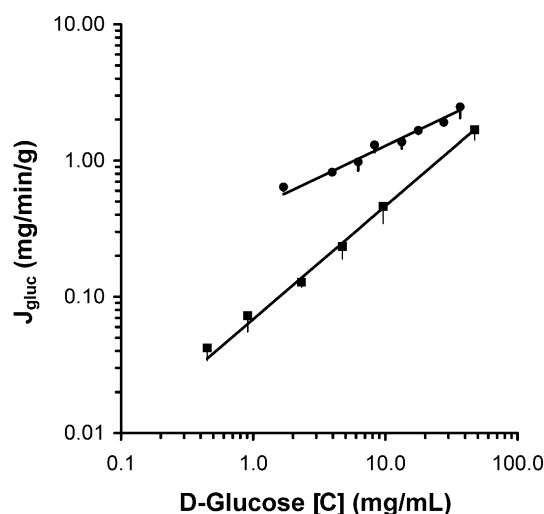


Fig. 3. Glucose flux in perfusate containing no initial NaCl (■), and 2.5 mg/ml NaCl (●) and mannitol with convection nullified. Each value is the mean (\pm S.E.) of three to six determinations. The lines are the least-squares regression fits. Glucose diffusion shows power law relationship with concentration-dependent permeability coefficient.

Table 2

Comparison of fractal power (FP) and Michaelis–Menten (MM) kinetic models

Kinetic model	Perfusate NaCl concentration (mg/ml)	R^2	AIC ^a
MM	0	0.9995	−39.5
FP	0	0.9997	−42.8
MM	2.5	0.9146	−10.1
FP	2.5	0.9720	−19.0

^a Akaike information criterion.

estimates did not reach a statistical level of significance. The typical MM parameters, the MM constant, K_m , and the velocity maximum, v_{\max} , were estimated by minimized least-squares regression analysis, and goodness-of-fit statistics compared (Table 2). The least-squares estimates of K_m and v_{\max} were 95.7 mg/ml and 5.09 mg/min/g, respectively, in the absence of initial sodium chloride in the perfusate; and, 11.7 mg/ml and 2.93 mg/min/g, respectively, in perfusate containing an initial sodium chloride concentration of 2.5 mg/ml. In both cases, the coefficient of determination, R^2 , was greater for the fractal power model and the AIC was less, indicating a superior model based on the observed data. Differences between models were more apparent in the presence of sodium chloride do to the greater curvature in the glucose flux–concentration relationship under these conditions. Furthermore, the reduction in K_m associated with the larger sodium chloride concentration is inconsistent with a competitive inhibitory MM model.

5.3. Glucose convection and diffusion

Glucose ultrafiltration was evaluated with sodium chloride perfusate concentrations of 2.5 and 7.5 mg/ml which resulted in large positive and negative free convection

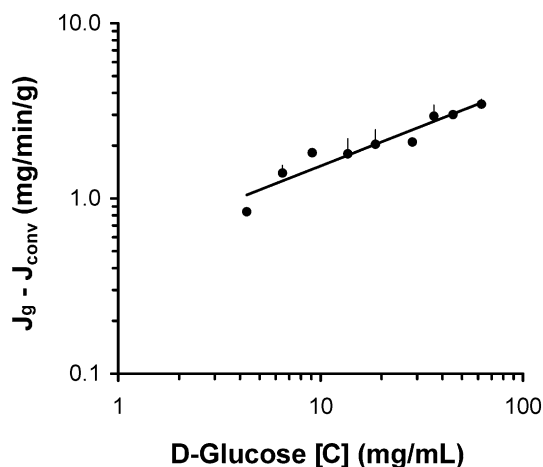


Fig. 4. Diffusive component of glucose flux resolved from total glucose mass transfer with free convection. Perfusate contains 2.5 mg/ml NaCl. Each value (●) is the mean (\pm S.E.) of three to six determinations. The line is the diffusive component of the least-squares best fit of the full model. $J_{\text{conv}} = \alpha J_v[C]$.

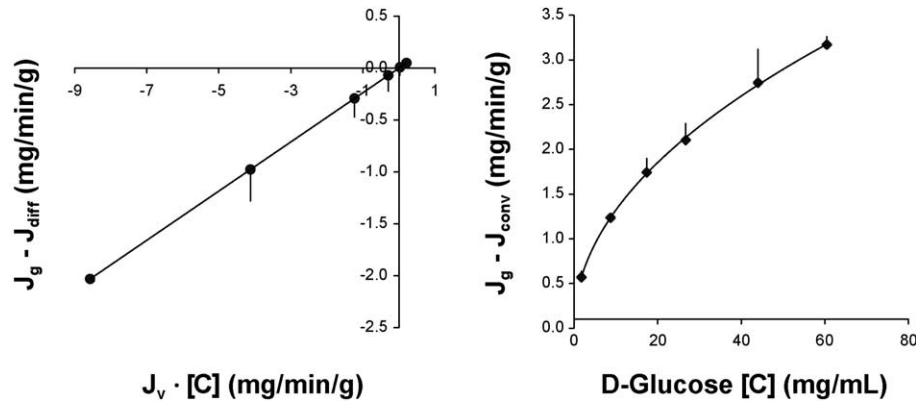


Fig. 5. Convective and diffusive components of glucose flux resolved from total glucose mass transfer with free convection. Perfusate contains 7.5 mg/ml NaCl. Each value is the mean (\pm S.E.) of three to five determinations. The line is the least-squares best fit corresponding to the aspect depicted from the full model.

currents. The convective–diffusive transport of glucose was best described by a three-parameter linear combination model in accord with Eq. (9). This model implies that glucose convection and diffusion pathways are independent. Total glucose mass transfer was resolved into its component parts, that resulting from ultrafiltration, and that resulting from diffusion (Figs. 4 and 5). No significant differences between descriptive transport parameters at 2.5 and 7.5 mg/ml sodium chloride concentrations were detected as evidenced by overlapping 90% confidence intervals (Table 3). In addition, no distinguishable differences were found between the diffusive component transport parameters in the convective–diffusive model and those parameter values when aqueous streaming was minimized. This finding indicates that convection currents do not intrinsically affect glucose diffusion. Based on the observed convection currents ranging from -142 to 155 $\mu\text{l/min/g}$ and the concordance of glucose diffusion, with and without aqueous streaming, the possibility that glucose diffusion is rate limited by a hydrodynamic barrier is mitigated since such a “stagnant” layer would be influenced by radial convection currents. These findings suggest that glucose mass transfer

coefficients are hydrodynamically stable in sodium chloride solutions containing 2.5–7.5 mg/ml.

The fractional contribution of convective glucose mass transfer was evaluated under proabsorptive and anti-absorptive conditions (Fig. 6). With a positive mucosal-to-serosal aqueous flux rate of 100 $\mu\text{l/min/g}$ and glucose average axial concentration of 13.6 mg/ml (15.0 mg/ml inlet concentration), maximum glucose convective transport was observed corresponding to $21 \pm 2.9\%$ of the total mass transfer. These results were observed with perfusate containing an initial 2.5 mg/ml sodium chloride concentration. When the perfusate contained an initial sodium chloride concentration of 7.5 mg/ml, resulting in largely negative aqueous flux rates, anti-absorptive mass transfer was observed. The maximum proabsorptive convective mass transfer occurred at 8.74 mg/ml average axial glucose concentration (10.0 mg/ml inlet concentration) and a positive aqueous flux rate of 24 $\mu\text{l/min/g}$ which contributed only $3.8 \pm 0.78\%$ of the total glucose mass transfer. Factors affecting the glucose convective transport rate were both the aqueous flux rate and the degree of ultrafiltration. The ultrafiltration coefficient, α , which is indicative of the relative convective velocity of glucose to

Table 3
Regression estimates for D-glucose mass transfer

Perfusate	Range of independent variables			Model parameters			R^2
	Sodium ion [C] (mg/ml)	D-Glucose [C] (mg/ml)	J_v ($\mu\text{l/min/g}$)	P^o (ml/min/g)	α	$1 - \beta$	
2.5 mg/ml NaCl	1.0; 1.3 ^a	4.3; 62	-92 ; 155	0.586 ± 0.1769^b (0.242, 0.930) ^c	0.331 ± 0.0702 (0.195, 0.468)	0.429 ± 0.0948 (0.245, 0.613)	0.8648
7.5 mg/ml NaCl	2.7; 3.0	1.8; 60	-142 ; 24	0.428 ± 0.1762 (0.387, 0.470)	0.237 ± 0.0091 (0.216, 0.258)	0.489 ± 0.0147 (0.454, 0.523)	0.9986
2.5 mg/ml NaCl rectified	1.1	1.7; 37	-12 ; 7.1	0.442 ± 0.0488 (0.348, 0.537)		0.462 ± 0.0364 (0.392, 0.533)	0.9720
0 mg/ml NaCl	0.25; 0.29	0.45; 47	-15 ; 13	0.0684 ± 0.00304 (0.0619, 0.0749)		0.831 ± 0.0119 (0.805, 0.856)	0.9997

^a Min; max.

^b Least-squares estimate \pm S.D.

^c Ninety percent confidence interval.

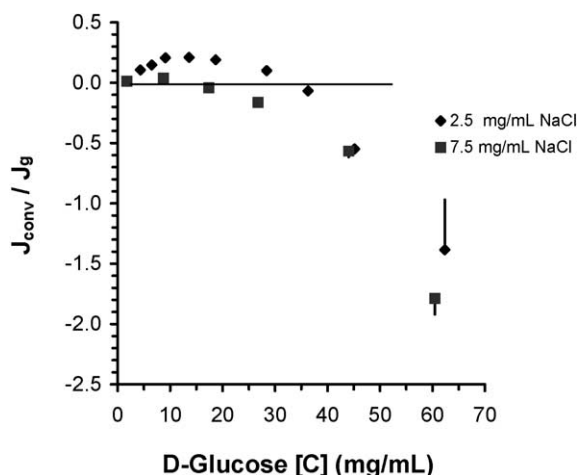


Fig. 6. Fractional mass transfer by glucose ultrafiltration in perfusates containing 2.5 or 7.5 mg/ml NaCl. Each value is the mean (\pm S.E.) of three to six determinations. Proabsorptive region corresponds with positive fractional values.

the aqueous stream velocity, was about 28%, showing a relative selective preference for water as compared to glucose.

6. Discussion

6.1. Inductive transport and hydraulic conductivity

Glucose, 3-*O*-methylglucose, and alanine have all been shown to markedly decrease transepithelial impedance [21] and resistance [22] to current carried by polyelectrolyte solutions. Sodium ion and creatinine fluxes increased in response to the reduced membrane resistance [22]. Water absorption is also markedly enhanced in vivo [14,19,23] in the presence of sodium chloride and glucose. Our results are consistent with these earlier findings, but also indicate that glucose and sodium chloride can act independently to increase hydraulic conductivity. Their mutual effect, however, significantly amplifies the rate at which water is absorbed and their quantitative interrelationship has been deduced. Of particular interest in this study, is the effect that mannitol has on attenuating the aqueous streaming current. Even in the presence of sodium chloride and glucose, the osmotic pressure gradient generated by the inclusion of mannitol in the perfusate can completely nullify the oncotic pressure gradient. This finding showing that reversal of the streaming current can be caused by rectification of physical forces implies that water absorption is the result of prevailing colligative properties of the intestinal mucosa and perfusate solution, and is not uniquely dependent on specific chemical composition, typical of selective carrier-mediated processes. These results preclude conventional notions that water absorption is dependent on a unique transporter (i.e. aquaporins), that the inductive hydraulic force is somehow related to a Na^+ -glucose biochemical

intermediate, or that aqueous streaming is induced by the glucose flux.

Although osmotic and oncotic forces were not found to specifically depend on sodium chloride and glucose, it was apparent that aqueous streaming was specifically related to sodium chloride and glucose concentrations. A crucial finding in this study was that in addition to the relationship between the aqueous flux and sodium chloride and glucose concentrations, glucose flux was dependent on sodium chloride concentration when subjected to isodynamic pressure and glucose chemical gradients. Similarly, sodium ion flux was dependent on glucose concentration when isodynamic pressure and sodium chloride gradients prevailed. Both glucose and sodium ion transepithelial mobility were increased in the presence of the auxiliary chemical component. These results suggest that the observed increase in hydraulic conductivity is a result of increased water mobility as well. In view of the ubiquitous increase in all transport mobilities measured, it may be inferred that the observed inductive effect is caused by the direct action of sodium chloride and glucose on the intestinal epithelium resulting in a reduction in transepithelial resistance. A direct interaction of sodium chloride and glucose on intestinal epithelia enhancing membrane permeability is concordant with reported in vitro results with regards to reductions in transepithelial impedance and resistance. Madera and Pappenheimer [24] have suggested an anatomical basis for changes in epithelial resistance. Alterations in membrane characteristics associated with transport properties, particularly diffusion, however, probably implicate changes in membrane features at a molecular level, although gross structural changes may occur as well.

6.2. Glucose mass transfer

Nonlinear diffusivities have been quantitatively modeled using power function relationships for a wide range of physical phenomena including plasma diffusion and thermal expulsion of liquid helium, diffusion of solutes in high-polymeric systems, and light scattering on semidilute polyelectrolyte solutions [25,26]. Here we have shown that the nonlinear transepithelial diffusion component of glucose mass transfer can be successfully modeled by considering a fractal power dependency of the permeability coefficient on glucose concentration. This was demonstrated under a variety of conditions including mass transfer in the absence of aqueous streaming and with free convection, and with different background sodium chloride concentrations. The fractal power dependency of the permeability coefficient is not consistent with the linear constraint on chemical species, intrinsic to the development of the MM quantitative kinetic relationship. This indicates that the notion of an epithelial transport intermediary, that is, a discrete transporter, cannot be supported by our data. Furthermore, although fractal power flux exhibits hyperbolic behavior, it is not asymp-

totic. This result is consistent with observed data from a large number of reported studies [27]. Power function diffusivity, however, bears resemblance to Freundlich-type absorptive behavior which is suggestive of underlying thermodynamic mechanisms.

The covariate dependency of glucose mass transfer on sodium chloride concentration was also demonstrated. The dependence of glucose mass transfer on sodium chloride concentration was most dramatically demonstrated when the perfusate did not contain any sodium chloride initially. In this case, large changes in mass transfer coefficients, P° and β , were observed. With initial sodium chloride concentrations ranging from 2.5 to 7.5 mg/ml, no statistically distinguishable changes in mass transfer coefficients, including α , were observed. These results may reflect a threshold dependency of membrane resistance on sodium chloride concentration and subsequent limiting change in resistance, or they may simply reflect limitations in the precision of our data and the ability to differentiate effects on mass transfer coefficients in this concentration range.

Although the diffusive component of glucose mass transfer represented by Eqs. (5) and (6) are expressed in first-order format with regard to the concentration gradient, a similar paradigm may be derived with the concentration gradient explicitly denoted as follows.

$$J_{\text{gluc, diff}} = -\frac{D^\circ}{C^\beta} \frac{\partial C}{\partial x} \quad (10)$$

When the convective mass transfer pathway is independent of the diffusive pathway, this equation may be directly integrated. After integration at a steady state, the flux is thus given by,

$$J_{\text{gluc, diff}} = \frac{D^\circ}{(1-\beta)\delta} C^{1-\beta} = P^\circ C^{1-\beta} \quad (11)$$

where C is the luminal (mucosal) concentration, δ the membrane thickness, and such that the down-stream concentration is considered negligible. Other paradigms may be expressed in terms of an activity gradient. Since these gradients are not accessible in a perfusion model, however, their values are difficult to assess and require further investigation using other epithelial membrane physical arrangements. It is not even clear whether the flux is dependent on a gradient corresponding with anatomical spacial attributes, an abstract gradient without anatomical limits, or by interfacial concentrations at the lumen/mucosa interface. Nevertheless, for comparisons sake, virtually all average axial glucose concentrations were greater than nominal values of glucose concentrations in rat plasma of about 0.6–0.8 mg/ml [28]. Furthermore, if one considers cerebral spinal fluid or aqueous humor of similar composition as that of intracellular or extracellular fluid, then with glucose values of about 0.4–0.6 mg/ml in these fluids, prevailing epithelial glucose concentrations may be considered as sink conditions. In addition, calculated values of D°

$\delta = P^\circ(1-\beta)$ from Eq. (11) corresponding to perfusates containing 2.5, 7.5, 2.5 (with mannitol), and 0 mg/ml sodium chloride (Table 3), are 0.25, 0.21, 0.20, and 0.057 ml/min/g. Comparatively, only when average axial sodium chloride concentration is minimal, is a large reduction in diffusivity observed. All average axial sodium ion concentrations were less than prevailing nominal values of sodium in plasma and other fluids of about 3.5 mg/ml.

In the context of recent findings by Lane et al. [18], we agree that the convective component of glucose mass transfer has only a minor effect on glucose absorption under most conditions. At a sodium chloride concentration of 7.5 mg/ml, near physiological salinity, only a maximal contribution of 4% was observed. However, under relatively narrow suitable conditions of glucose concentrations of about 5–20 mg/ml and a sodium chloride concentration of 2.5 mg/ml, up to 21% of glucose mass transfer was accounted for by convection. These conditions appear to correspond to those required for maximum mobility of glucose, water, and sodium ion, and at which maximal aqueous streaming occurs. Although, under most circumstances, the fractional contribution of convective glucose mass transfer may be relatively unimportant, it is critical to isolate and characterize this component of mass transfer to understand and quantify the diffusive component.

In conclusion, we have found that perfusate chemical composition has a profound effect on the mass transfer of glucose and water. In view of the effect that perfusate composition has on glucose and sodium ion mass transfer under isodynamic conditions, nonlinear concentration-dependent mass transfer probably arises as the result of the direct effect of glucose and sodium chloride on epithelial resistance. Although in situ perfusion approaches do not permit the evaluation of a molecular basis for changes in epithelial mobility, molecular studies may lead to a better understanding between the interaction of a mobile molecule and a resistive membrane provided that changes in chemical composition of the membrane can be correlated with changes in physical properties. Based on the results of this study, glucose mass transfer can be effectively quantified by a glucose concentration-dependent fractal power model in the presence of a saline background.

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