Intestinal Water and Solute Absorption Studies: Comparison of *in Situ* Perfusion with Chronic Isolated Loops in Rats

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The effects of lumenal glucose on jejunal water transport and the influence of glucose-induced water absorption on solute uptake from single-pass perfusions are compared in anesthetized rats *in situ* and isolated chronic loops in unanesthetized rats *in vivo*. While the magnitudes of solute membrane permeabilities are consistently higher in the chronic loop system, the effects on water transport and its promotion of jejunal solute uptake are comparable between the two experimental systems. The effect of glucose-induced water absorption on the enhanced/baseline jejunal uptake ratio of the hydrophilic drug, acetaminophen, is greater than that for the lipophilic drug, phenytoin, in both experimental systems. The fact that chronic loop effective solute permeabilities were equivalent to solute membrane permeabilities *in situ* is consistent with greater lumenal fluid mixing *in vivo*. In addition, *in situ* body temperature affects the uptake of phenytoin but not acetaminophen, water, or glucose. This suggests that active and paracellular solute transport is not compromised *in situ*, while membrane partitioning and diffusion of lipophilic species are more sensitive to experimental conditions.

KEY WORDS: intestinal drug and water absorption; intestinal perfusion; glucose; acetaminophen; phenytoin.

INTRODUCTION

Isolated intestinal perfusions in anesthetized rats have been carried out (1) to assess drug and nutrient absorption at the level of the intestinal membrane under near physiological conditions. The *in situ* method bypasses drug dissolution and stomach emptying steps after oral dosing while affording input control and choice of perfused intestinal region. It has advantages over *in vitro* methods by providing intact lymphatic and blood circulation for solute uptake with extended tissue viability.

Solute uptake from a variety of perfusion techniques (flow patterns) in the *in situ* system was reported previously (2). *In situ* studies of solute intestinal permeability dependence on perfusion hydrodynamics (3), the drug membrane/water partition coefficient (4), and intestinal water transport (5) have enhanced our understanding of processes controlling intestinal drug absorption. In addition, correlations of the fraction dose absorbed in human *in vivo* studies with rat jejunal drug permeabilities suggest that this experimental system may provide an intestinal membrane-level screen for drug absorption potential (6).

A primary limitation of the *in situ* technique is the difficulty in obtaining multiple permeability measurements in the same experimental animal to compare solutes or input conditions. *In situ* treatment comparisons require sizable animal numbers to demonstrate permeability differences because of animal-to-animal variability. Furthermore, if absorption is to be assessed by pharmacologic response, anesthesia may complicate data analysis. The development of the rat chronic isolated loop technique permits repeated perfusion studies in the same unanesthetized experimental animal (7).

In this study, rat jejunal water flux was determined as a function of perfusate composition in the *in situ* and chronic loop systems under equivalent input conditions, and the effect of glucose-induced jejunal water absorption on the passive uptake of a hydrophilic and lipophilic solute was compared in these two systems.

MATERIALS AND METHODS

Materials

Potassium chloride, sodium chloride, 2(*N*-morpholino)ethanesulfonic acid (MES), polyethylene glycol 4000 (PEG 4000), dibasic sodium phosphate, citric acid, D-glucose, 3-oxymethyl glucose, mannitol, acetaminophen (4-acetamidophenol), and phenytoin (5,5-diphenylhydantoin) were of analytical grade from Sigma Chemical Co. (St. Louis, MO). Carbon 14-polyethylene glycol (sp act, 3.2×10^6 dpm/μmol) was used as a nonabsorbable marker for net water flux. Tritiated water (sp act, 5.6×10^7 dpm/ml) was used for determination of water absorption (influx). Radiolabeled compounds were obtained from New England Nuclear (Boston, MA).

Perfusion Solutions

Buffer solutions used for jejunal perfusions included a modified MES buffer adjusted to 290 ± 20 mOsm/kg with either sodium chloride, choline chloride, or mannitol and adjusted to pH 6.5 ± 0.1 with sodium hydroxide. Modified MES buffer contained 5 m*M* KCl, 100 m*M* NaCl, 10 m*M* MES, and 0.01% PEG 4000 traced with carbon 14-PEG 4000. Modified McIlvaine buffer was prepared with 137 m*M* dibasic sodium phosphate and 31.4 m*M* citric acid, pH 6.5 ± 0.1, adjusted to 290 ± 20 mOsm/kg with sodium chloride. Unbuffered demineralized water was adjusted to pH 6.5 ± 0.1 just prior to experimental perfusion. Whenever a buffered monosaccharide solution was prepared, the sugar quantitatively replaced sodium chloride in terms of osmotic equivalents in the control buffer.

Surgical Preparations

In situ external jejunal perfusions were carried out in fasted male Sprague–Dawley rats weighing 300–350 g as outlined in a previous publication (5). Following induction of anesthesia, rats were placed on a heating pad under a surgical lamp to maintain body temperature. In some of these

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studies, rectal, exterior, and interior abdominal body temperatures were measured. To study the effect of temperature, a separate treatment group was isolated without maintenance of body temperature via heating pad and surgical lamp. A 6- to 8-cm jejunal segment was cannulated at the distal end with a short segment of Teflon tubing positioned to drain into collecting tubes. In some of these studies a temperature probe (Yellow Springs Instrument Co.) was inserted into the intestinal lumen through the distal cannula to monitor jejunal temperature. This same segment was cannulated at the proximal end with a small glass entry tube tied in place externally with surgical thread. The segment was maintained outside the abdominal incision and the entire surgical area covered with parafilm to minimize temperature reduction through evaporation.

In vivo chronically isolated internal jejunal loop perfusions were carried out (with minor modifications) according to Poelma and Tukker as detailed in a previous report (7). Following induction of anesthesia, a jejunal segment of approximately 6–8 cm was isolated maintaining intact mesenteric circulation. The loop was attached to the abdominal wall through the placement of stainless-steel proximal and distal cannulae for external input and collection, respectively. The continuity of the remaining intestinal tract was restored by end-to-end anastomosis and returned to the abdominal cavity. Subsequent to abdominal suture and surgical recovery (usually 3–4 days), the rat was restored to normal dietary intake and considered ready for perfusion experiments.

Perfusion Technique

In situ perfusion solution was pumped through inlet Teflon tubing water-jacketed at 37°C prior to entering the jejunal segment at a flow rate of 0.2 ml/min (Harvard Perfusion Pump Model No. 31). The jejunal segment was routinely prewashed at 0.7 ml/min with drug-free buffer prior to the initiation of perfusion treatments. Outlet tubing samples were collected every 10 min. Analysis showed that steady-state water and solute transport were established by 30–40 min and mean steady-state data over five collection periods were used to report water flux and solute permeability.

Chronically isolated jejunal loop perfusions were performed following rinsing of the loop at 0.7 ml/min with isotonic saline for 15 min in order to clear the intestinal segment of excess mucus. Perfusion solutions were pumped through the loop at a flow rate of 0.2 ml/min maintained by a Harvard syringe pump (Model No. 22). Steady-state water and solute transport were established in 10 min in this system. The mean transport values were obtained from three or four samples collected at steady state. The choice of perfusion flow rate in both systems is consistent with gastric emptying of a glucose load in rats at the caloric density chosen for the perfusion experiments (5).

Analytical Methods

Net water transport was determined by change in concentration of the nonabsorbable marker, 1,2-[^{14}C]PEG 4000 from inlet (i) and outlet (o) perfusate samples. Scintillation counting was carried out in Ecolite cocktail (ICN Biomedicals, Inc., Irvine, CA) on a Beckman LS9000 (Beckman Instruments, Irvine, CA). Net water flux (μl/min-cm) was calculated at a given flow rate, Q_i (μl/min), per unit of perfused intestinal length, L (cm), as carbon-14 disintegrations per minute using Eq. (1).

$$J_{\text{net flux}} = (^{14}\text{C dpm}_{\text{o-i}} \times Q_i)/(^{14}\text{C dpm}_{\text{o}} \times L) \quad (1)$$

Outlet PEG 4000 concentration less than inlet concentration, implying net water secretion into the jejunal lumen, is represented as negative net water flux values. Outlet PEG 4000 concentration greater than inlet concentration, implying net water absorption from the jejunal lumen, is represented as positive net water flux values.

Water influx (lumen-to-mucosa water flux as μl/min-cm) was obtained through measurement of tritiated water loss from inlet to outlet (8) and computed using Eq. (2). It was assumed that the efflux of labeled water from the mucosa back to the lumen was negligible.

$$J_{\text{influx}} = (^{3}\text{H dpm}_{\text{i}} \times Q_i - {}^{3}\text{H dpm}_{\text{o}} \times Q_o)/(^{3}\text{H dpm}_{\text{i}} \times L) \quad (2)$$

Effluent flow rates (Q_o) represents the difference between the perfusion flow rate (Q_i) and the product of net water flux and perfused intestinal length ($J_{\text{net}} \times L$). Water efflux from mucosa to lumen is calculated as the difference between water influx and net water flux.

Solute absorption was evaluated as the rate of steady-state drug loss from perfusion solution output corrected for net water flux by $[(C_o - C_m)/C_o]_{\text{drug}} \times (C_m/C_o)_{\text{PEG}}$, where C_o and C_m represent perfusion input and output concentrations, respectively. Solute concentrations from inlet and outlet perfusate were determined using a reverse-phase high-performance liquid chromatographic (HPLC) system consisting of a WISP 710B autoinjector (Millipore, Milford, MA), a Waters Model 501 solvent pump, and a Spectroflow 773 absorbance detector (Kratos, Ramsey, NJ). The mobile phase for acetaminophen was a 15:85 mixture of methanol–water (v/v) and theophylline was used as an internal standard. The flow rate was 1.2 ml/min and the ultraviolet (uv) absorbance was measured at 254 nm. The mobile phase for phenytoin was a 55:45 mixture of methanol–water (v/v) and *p*-methylphenylhydantoin was used as an internal standard. The flow rate was 1.2 ml/min and the uv absorbance was measured at a wavelength of 228 nm.

Data Analysis

Water flux and solute permeabilities are represented as the mean ± standard error (SE) for n experiments. An unpaired t test was used to compare mean values between treatments for groups of rats in the *in situ* studies (n = 4–6 rats), while a paired t test was used to compare treatments in the same chronic loop animals (n repeated studies in two rats). Statistical differences were assessed at a 5% level of significance and analysis of variance was performed to evaluate the level of interaction between treatment variables.

RESULTS

Baseline Perfusions

Since the influence of intestinal water flux on solute

uptake was a major focus of the experimental comparison, baseline water flux from perfusion solutions containing various buffer components and osmotic agents was evaluated in this study. All buffered perfusions were carried out with isoosmotic (290 ± 20 mOsm/kg) solutions at pH 6.5 ± 0.1. Jejunal water flux from isoosmotic McIlvaine (anionic:citrate-phosphate) and MES (zwitterionic:2-*N*-morpholinoethanesulfonic acid) was compared with previous studies performed *in situ*.

In previous studies characterizing baseline water flux in the *in situ* perfusion system, Krebs–Henseleit (bicarbonate) buffer at pH 7.4 generated considerably more temporal variation in water transport than did either McIlvaine or Sorenson's phosphate buffer (9). In more recent studies (5), perfusion of MES buffer at pH 6.5 resulted in the generation of minimal steady-state jejunal water transport *in situ*. Average steady-state water influx and efflux in chronic loop versus acute *in situ* perfusion are compared in Table I for these two buffers at pH 6.5 and a perfusion flow rate of 0.2 ml/min. Both McIlvaine and MES buffer generated net water secretion in acute *in situ* and chronic loop *in vivo* studies, with MES producing less net water transport in both systems (Table I).

A comparison of net jejunal water flux from sodium chloride, choline chloride, and mannitol osmolality adjustment in the chronic loop system is shown in Table II. A recent report has documented that oral mannitol (at 55 mOsm/kg) suppresses intestinal solute uptake *in vivo* (10). In accordance with results from these more isolated systems, there is no evidence that this effect is mediated by significant changes in jejunal water transport. While only two experiments were performed with choline chloride as an osmotic agent, larger differences in water influx and efflux (possibly the result of local cholinergic effects) suggest that this salt may not be an appropriate osmotic agent for intestinal transport studies.

While day-to-day within animal variability in chronic loop baseline water transport was not significant, depressed water influx and resultant net water secretion were consistent with the manifestation of diarrhea (Table III). This baseline secretion is reversed by sodium chloride/D-glucose solutions in these animals as has been previously established and utilized in oral rehydration therapy to treat severe diarrhea (8).

Glucose-Induced Water Absorption

Net jejunal water absorption was generated by perfusion of either lumenal isotonic D-glucose or hypotonic unbuffered water as compared to isotonic buffers in the chronic loop system. Interestingly, water efflux was depressed by hypotonic perfusion of unbuffered water, while lumenal glucose did not depress water efflux in the rat prone to diarrhea (Fig. 1). Water influx generated by isotonic D-glucose/NaCl/MES was enhanced over perfusion with isotonic NaCl/MES, promoting net water absorption in the diarrheal animal. Isoosmotic pH 6.5 MES/112 m*M* D-glucose significantly increased net jejunal water absorption over baseline by 2.8 ± 0.82 μl/min-cm (Fig. 2) in chronic loop rats. Significant net water absorption (3.4 ± 0.38 μl/min-cm) could also be generated by perfusion of unbuffered water. However, jejunal perfusion of water does not represent a physiologically relevant input since fluid entering the jejunum *in vivo* will be isoosmotic or slightly hyperosmotic under certain fed-state conditions (5). In addition, comparable net water absorption could not be generated by perfusion of the nonmetabolizable monosaccharide 3-oxymethyl glucose *in situ* (Fig. 3), a substrate for the same sodium-dependent mucosal carrier as D-glucose. Comparison of water flux data generated by isoosmotic D-glucose between the *in situ* and the *in vivo* perfusion techniques shows that net water flux results are equivalent in both the *in vivo* chronic loop and the *in situ* perfusion system (Table IV).

Solute Uptake

In the *in situ* system, solute membrane permeabilities (P_m) are determined by factoring out a theoretically determined aqueous resistance (R_{aq}) to solute transport (11). Aqueous resistance is calculated by using a laminar flow model established by residence time analysis for the rat *in situ* perfusion system (3). The total effective resistance (R_{eff}) to solute transport is evaluated from water-corrected solute output over input concentration ratios.

$$R_{eff} = R_{aq} + \text{membrane resistance} \quad (3)$$

Corresponding effective, aqueous, and membrane permeabilities are the reciprocals of these respective resistances.

$$\text{permeability} = 1/\text{resistance} \quad (4)$$

Table I. Comparison of MES and McIlvaine Water Flux in Two Perfusion Techniques: *In Situ* Perfusion vs Isolated Chronic Loop Perfusion[a]

Experimental design	Buffer system	Water influx (μl/min-cm)	Water efflux (μl/min-cm)	Water net flux (μl/min-cm)[b]
In situ	MES	10.46 ± 0.19 (2)	10.64 ± 0.18 (2)	−0.19 ± 0.01 (2)
In situ	McIlvaine	—	—	−0.60 ± 0.09 (6)[c,*]
Chronic isolated loop	MES	10.61 ± 0.23 (9)	10.72 ± 0.24 (9)	−0.11 ± 0.22 (9)
Chronic isolated loop	McIlvaine	10.79 ± 0.60 (3)	12.04 ± 0.34 (3)	−1.26 ± 0.27 (3)**

[a] Data are expressed as mean ± SE (number of experiments).
[b] Positive sign indicates net water absorption; negative sign indicates net water secretion.
[c] Data are from the work of Meadows (8).
* Data are statistically different ($P < 0.005$) from MES buffer perfused *in situ* loop.
** Data are statistically different ($P < 0.05$) from MES buffer perfused in chronic isolated loop.

Table II. Comparison of Osmotic Agents in Chronic Isolated Loop[a]

Buffer content	Water influx (μl/min-cm)	Water efflux (μl/min-cm)	Water net flux (μl/min-cm)[b]
MES/sodium chloride	10.61 ± 0.23 (9)	10.72 ± 0.24 (9)	−0.11 ± 0.22 (9)
MES/choline chloride	9.33 ± 0.46 (2)	10.27 ± 0.35 (2)	−0.96 ± 0.10 (2)
MES/mannitol	9.42 ± 1.04 (3)	9.90 ± 1.16 (3)	−0.48 ± 0.30 (3)

[a] Data are expressed as mean ± SE (number of experiments).
[b] Positive sign indicates net water absorption; negative sign indicates net water secretion.

The effective permeability is determined by

$$P_{eff} = Q(1 - C_m/C_o)/2\pi RL \quad (5)$$

where C_o represents inlet solute concentration and C_m represents water transport-corrected outlet solute concentration determined experimentally. Q is perfusion flow rate, L is the length of the perfused intestinal segment, and R is the inner radius of the segment. Drug C_m is corrected for intestinal water transport (net absorption or secretion) by normalizing for nonabsorbable marker (PEG 4000) outlet to inlet concentration ratio. Data are represented as dimensionless (*) quantities obtained by normalizing permeabilities for the ratio of intestinal radius to solute aqueous diffusivity.

Solute membrane permeabilities (P_m) proved to be consistently higher in the chronic loop system that *in situ*. However, effective solute permeabilities (P_{eff}) in the chronic loop more closely matched membrane solute permeabilities (P_m) *in situ*. When the hydrophilic drug, acetaminophen, was perfused in these two experimental systems, the dimensionless membrane permeability (P_m*) in the *in situ* system was 0.6 ± 0.2 (n = 4), while the dimensionless effective permeability (P_{eff}*) in the chronic loop system was 0.9 ± 0.1 (n = 4). When the lipophilic drug, phenytoin, was perfused in both systems, P_m* *in situ* was 4.4 ± 0.4 (n = 8), while chronic loop P_{eff}* was 4.7 ± 0.2 (n = 8).

Furthermore, acetaminophen permeability was shown to increase significantly when coperfused with isoosmotic D-glucose and to the same extent in both systems [P_m* increased from 0.6 to 1.4 *in situ* (5) and P_{eff}* increased from 0.9 to 1.9 *in vivo* (Fig. 4)]. For the more lipophilic phenytoin, the increase in mean permeability in the presence of isoosmolar D-glucose was not statistically significant in either system at the perfusion flow rate used in these studies. This result is at odds with a previous report (12) documenting an *in situ* glucose effect on phenytoin uptake at a considerably higher perfusion flow rate.

In light of the fact that baseline and glucose-induced jejunal water transport were equivalent in both experimental systems, additional studies were conducted to discern the basis for higher solute membrane permeabilities in chronic loop versus *in situ*. While chronic loop lumenal conditions are maintained at body temperature, failure to maintain rat body temperature *in situ* results in temperature drops within the perfused intestinal lumen of up to 4°C (Table V). An *in situ* comparison of water and solute transport with and with-

Table III. Day-to-Day Comparison of MES Buffer Water Flux in Rat Chronic Isolated Jejunal Loop

Rat code	Water flux (μl/min-cm)	Date 1	2	3	4	5
A	Influx	11.13	11.80	10.62	11.16	9.90
	Efflux	10.43	11.13	10.60	11.77	9.60
	Net flux[a]	0.70	0.67	−0.02	−0.61	0.31
B	Influx	8.02[b]	10.33	9.57	10.77	10.14
	Efflux	10.07[b]	11.74	10.09	10.93	10.16
	Net flux[a]	−2.05[b]	−1.41	−0.52	−0.16	0.02

[a] Positive sign indicates net water absorption; negative sign indicates net water secretion.
[b] Rat B had diarrhea.

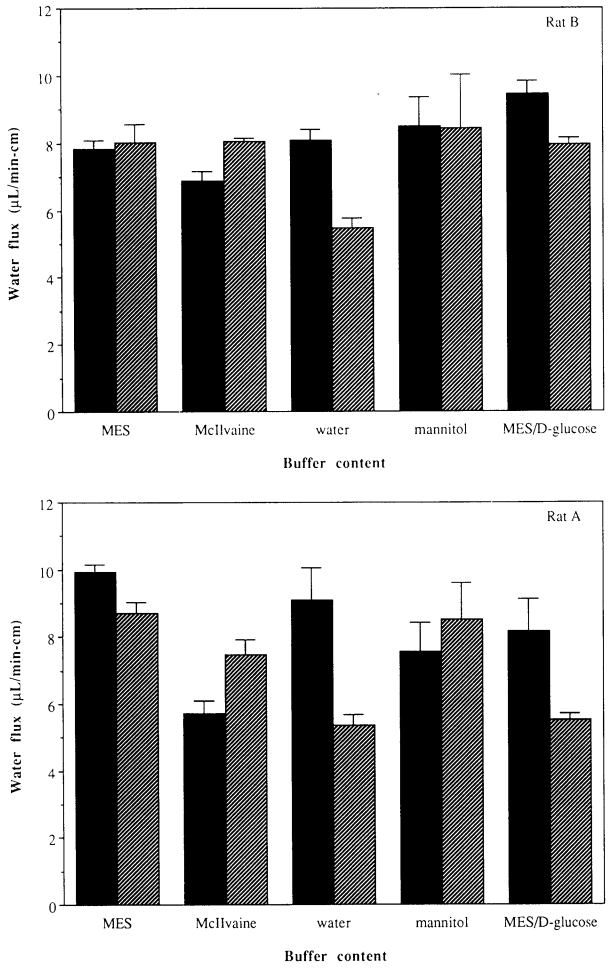

Fig. 1. Mean water efflux, influx, and net flux in two chronic jejunal loop rats as a function of perfusate composition. All are isotonic except water. Means and standard errors are from four experiments in each rat. Rat B suffered chronic diarrhea. (■) Influx; (▨) efflux.

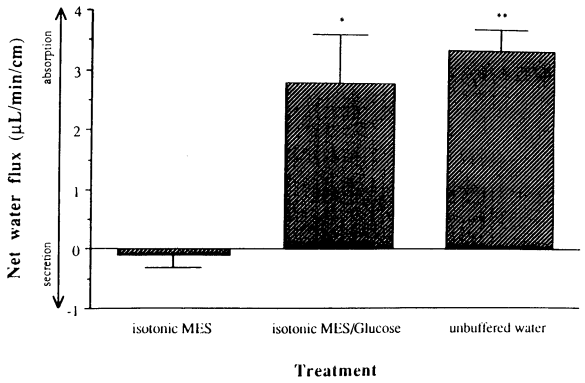

Fig. 2. Comparison of net jejunal water flux from perfusion of isotonic MES buffer, isotonic MES/112 m*M* D-glucose, and unbuffered water in the chronic isolated jejunal loop system. Data are presented as mean ± SE (n = 3). (*,**) Net water flux was significantly different at a t-test P value <0.001.

out temperature control indicated no significant differences in glucose-induced water flux, glucose effective permeability (from lumenal concentrations of 42 and 84 m*M*), and acetaminophen effective permeability (from lumenal concentrations of 0.2 m*M*). In contrast, phenytoin effective permeability was significantly higher in rat perfusion studies at concentrations of 80 μ*M* in which lumenal temperature was maintained equivalent to body temperature (Table V).

In experiments performed to assess the extent of lumenal mixing in chronic loop versus *in situ* experiments, perfusions at high flow rates (0.8 ml/min) were conducted to detect oscillations in output fluid weight over short time collection intervals (25 sec). A more pronounced oscillation pattern was observed in unanesthetized *in vivo* chronic loop rats than in anesthetized rats perfused *in situ* (Fig. 5). The more pronounced oscillations are hydrodynamically consistent with motility-generated intestinal mixing. (Steady outputs

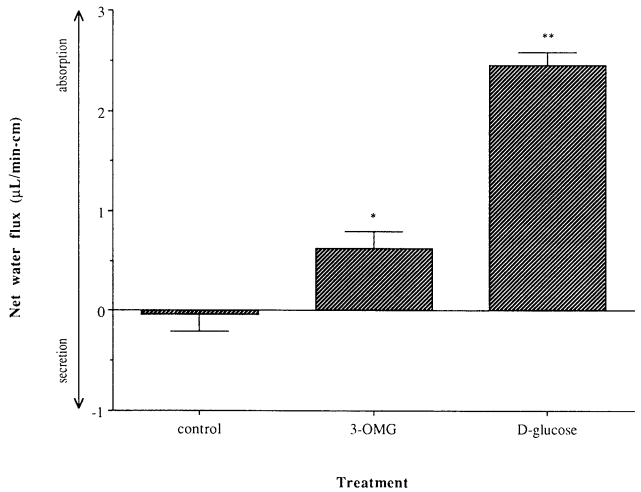

Fig. 3. Comparison of net jejunal water flux from perfusion of isotonic MES buffer, isotonic MES/112 m*M* 3-oxymethyl glucose, and isotonic MES/112 m*M* D-glucose in the *in situ* system. Data are presented as mean ± SE (n = 6, 11, and 10 for isotonic MES, isotonic MES/3-oxymethyl glucose, and isotonic MES/D-glucose, respectively). (*,**) Net water flux was significantly different at a t-test P value <0.001.

Table IV. Effect of D-Glucose-Induced Net Water Transport in the *in Situ* and Chronic Isolated Loop

Experimental design	Glucose (m*M*)	Net water flux (μl/min-cm)[ab]
In situ	112	2.44 ± 0.18 (4)
Chronic isolated loop	112	2.80 ± 0.82 (3)

[a] Data are expressed as mean ± SE (number of experiments).
[b] Positive sign indicates net water absorption; negative sign indicates net water secretion.

collected from input tubing verified that oscillations were generated by the perfused intestinal segment and not by perfusion pump fluctuations.) These patterns are damped out when output sampling is carried out over time intervals exceeding intestinal residence time (approximately 1 min at this high flow rate). Oscillatory output is minimized under laminar flow conditions established for *in situ* perfusions (3). Significant changes could not be discerned in output oscillation as a function of the depth of anesthesia *in situ* (Fig. 5).

DISCUSSION

Acetaminophen and phenytoin were selected as "passively absorbed" solutes for two reasons: first, to compare baseline solute permeability differences as a function of drug lipophilicity between *in situ* and *in vivo* experimental perfusion systems; and second, to compare the effect of glucose-induced water absorption on solutes for which intestinal membrane transport is likely dominated by different transport pathways [phenytoin/transcellular and acetaminophen/paracellular (5)].

Solute membrane permeabilities were higher in perfusion studies in chronic loop *in vivo* than *in situ*. Since solute membrane transport parameters are temperature dependent in theory, it might be anticipated that lower lumenal temperatures in the *in situ* system would generate *in situ–in vivo* membrane permeability differences. However, failure to

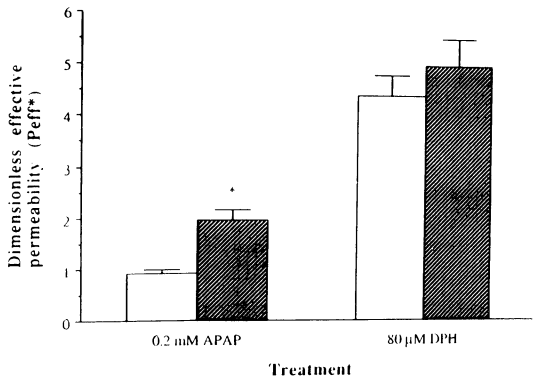

Fig. 4. Acetaminophen and phenytoin dimensionless effective permeabilities (P_{eff}*) in steady-state rat chronic isolated jejunal loop perfusion as a function of D-glucose-induced water absorption. Acetaminophen was perfused at a concentration of 0.2 m*M*, while phenytoin was perfused at a concentration of 80 μ*M* with or without 112 m*M* D-glucose. Results are presented as mean ± SE (n = 3). (*) Permeability was significantly different at a t-test P value <0.005. (□) Control; (▨) with D-glucose.

Table V. *In Situ* Dimensionless Effective Jejunal Permeabilities of Acetaminophen (APAP), D-Glucose (DG), and Phenytoin (PHT) and Jejunal Glucose-Induced Water Flux in Temperature (*T*)-Controlled and Noncontrolled Rats[a]

Treatment	Lumenal *T* (°C)	Net water flux (μl/min-cm)	P_{eff}*		
			APAP	DG	PHT
T controlled	38.7 ± .25	3.4 ± .57	2.0 ± .22	2.5 ± .33	3.6 ± .42
Noncontrolled	34.3 ± .45	2.5 ± .51	1.9 ± .20	2.1 ± .18	2.3 ± .37

[a] Data are shown as mean ± SE. Phenytoin effective permeability was significantly different as a function of treatment at a *t*-test *P* value <0.05.

maintain lumenal temperature *in situ* equivalent to body temperature *in vivo* did not significantly influence jejunal transport of D-glucose from perfusions at high concentration (42 and 84 m*M*) or the transport of acetaminophen from perfusion concentrations of 0.2 m*M*. While temperature control did promote higher mean water flux as stimulated by lumenal glucose, this effect was also not statistically significant. Maintenance of lumenal temperature did promote higher permeability of the lipophilic phenytoin. A plausable hypothesis is that mucosal membrane lipid fluidity is depressed at lower lumenal temperature, reducing the rate of lipophilic drug membrane transport. An alternative mechanism, temperature influences on villus and/or mesenteric blood flow (to provide sink conditions for this well-absorbed drug), would likely have promoted glucose uptake to the same extent as for phenytoin (13).

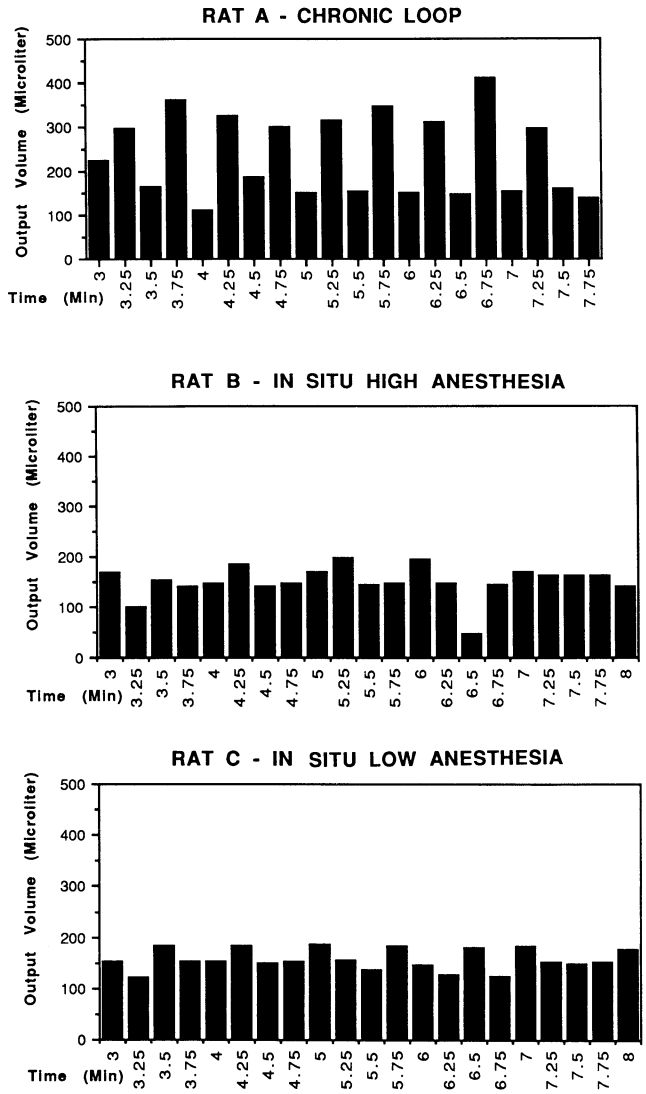

Fig. 5. Perfusate output volume over 15-sec time intervals from 3 to 8 min of perfusion at 0.8 ml/min in chronic isolated loops (A) and *in situ* at high (B) and low (C) anesthesia levels. Volume output from the perfusion pump was invariant over these same time intervals.

The magnitude of glucose-induced water absorption was equivalent *in vivo* and *in situ*. Similar effects of water absorption on the transport of the low molecular weight, hydrophilic drug, acetaminophen, suggests that solvent drag of solute through the paracellular pathway operates to a comparable extent in both perfusion systems. This finding indicates that differences in water transport between the two experimental systems do not account for the higher solute membrane permeabilities observed in chronic loops versus *in situ*.

Since chronic loop solute effective permeabilities equate with solute membrane permeabilities *in situ*, it is most likely that aqueous resistance to solute transport is lower *in vivo* than *in situ*. Residence time analysis experiments confirming laminar flow hydrodynamics *in situ* were originally performed at input perfusion flow rates of 0.5 ml/min (3). Previous *in situ* perfusions with phenytoin were carried out at this same flow rate in order to minimize aqueous resistance control of drug transport (12). The experiments conducted in this system comparison study were performed at a lower flow rate (0.2 ml/min), consistent with gastric emptying of an oral glucose load *in vivo* (14). At this lower flow rate, aqueous boundary layer resistance will dominate membrane transport of the lipophilic phenytoin and solvent drug effects near the membrane should be inconsequential.

Aqueous permeability calculations were performed assuming similar laminar flow hydrodynamics in both experimental perfusion systems. Output volume measurements at high flow rates over short time intervals suggest that better mixing occurs in the chronic loop system than *in situ* as has been reported by other researchers (15). Further investigations utilizing detailed residence time analysis coupled with hydrodynamic modeling to fit nonabsorbable marker output data should permit an assessment of the significance of lumenal fluid mixing in chronic loops. *In situ* observations of peristaltic movements in the isolated intestinal segment indicate that some mixing occurs in this system. However, these movements are of a short duration and seemingly random as a function of perfusion treatment conditions such as

the depth of anesthesia. It is not unreasonable to postulate that a motility response to perfusion occurs in the alert chronic loop rat that is not present to the same extent in the exterior jejunal loop of the *in situ* anesthetized rat. The fact that steady-state absorbable and nonabsorbable solute output-to-input concentration ratios are established more rapidly in the chronic loop system provides further evidence for better mixing.

This study confirms a previous finding *in situ* that glucose-induced water absorption enhances the jejunal uptake of a small hydrophilic solute to a greater extent than a more lipophilic solute (5). The extents of glucose-induced water transport *in situ* and *in vivo* were comparable, as were the extents to which acetaminophen permeability was enhanced. The permeability of phenytoin was also enhanced by glucose in both experimental systems. However, its lipophilicity and corresponding membrane permeability are sufficiently high that solvent drag through paracellular pathways is insignificant compared to drug transcellular uptake. Dimensionless membrane drug permeabilities in rat perfusions higher than one have been correlated with 100% absorption from oral solution administration in humans (6). In this regard, the data suggest that glucose-induced water absorption might enhance the absorption of small hydrophilic drug molelcules possessing poor membrane permeability. This glucose effect should be of little consequence for lipophilic drugs, for which dissolution rate rather than membrane permeability dominates drug absorption variability.

In summary, solute and water transport are comparable in the *in situ* and chronic loop systems. However, better mixing in chronic loops suggests that the laminar flow boundary layer analysis used to factor out aqueous resistance *in situ* may not provide an appropriate model *in vivo*. Several other prominent distinctions between these experimental systems are worthy of note. While jejunal water transport does not appear to be affected by anesthesia, measurements of solute uptake may be a function of the animal's state of consciousness. This may be important when drug absorption is evaluated from plasma levels and will likely depend on anesthetic state when absorption is assessed from pharmacologic response. A primary advantage of the chronic loop system is the capacity to perform treatment comparisons from serial drug absorption studies in which the animal functions as its own control. However, in these limited experimental studies, chronic isolated loop rats displayed between-animal variability that is likely a strong function of recovery from surgery and general health conditions. In addition, the maintenance of chronic loop animals requires significant outlay of time, while the *in situ* studies require more experimental animals per data point. A primary advantage of the *in situ* system is the availability of a mesenteric blood sampling compartment during perfusion, while systemic blood sampling can be performed in either system. In conclusion, the two systems demonstrate comparable water and solute transport. Both systems have advantages and disadvantages and system choice may be motivated by the method of solute absorption measurement.

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