Absorption of D-Glucose in the Rat Studied Using *In Situ* Intestinal Perfusion: A Permeability-Index Approach

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Purpose. A permeability-index approach was developed and used to study the transport of D-glucose in the jejunum and ileum of rats.
Methods. The effective permeability coefficient (P_e) of [^{3}H]D-glucose and [^{14}C]antipyrine (an internal standard) in jejunum and ileum of four rats was determined using an *in situ* rat intestinal perfusion technique. The permeability ratio of the test compound (D-glucose) to the internal standard was defined as the permeability-index (P_i), which was mathematically independent of the length and surface area of the intestinal segment perfused. Using this approach, the transport of [^{3}H]D-glucose in jejunum and ileum of eight animals was investigated at concentrations ranging from 1 to 300 mM. The tissue/perfusate distribution of [^{3}H]D-glucose and [^{14}C]antipyrine at steady state was also determined.
Results. The variability (%CV) in P_i of D-glucose was only ~5%, compared with 23–36% in P_e values of D-glucose or antipyrine alone. The permeability and tissue distribution of [^{14}C]antipyrine were unaffected by the presence of D-glucose. In contrast, the permeability and tissue distribution of [^{3}H]D-glucose were concentration-dependent in both jejunum and ileum. The transport of D-glucose was studied assuming that the transport was mediated by a carrier (with maximum flux, V_{max} and dissociation constant, K_m) as well as by non-saturable transport (P_d). The maximum transport capacity for D-glucose in jejunum (0.522 μmole/min/cm^2) was twice that in ileum (0.199 μmole/min/cm^2), but the affinity ($1/K_m$) was less than half of that in ileum (1/(48.2 μmole/mL) vs. 1/(21.4 μmole/mL)), rendering a similar active transport efficiency (V_{max}/K_m) in these two regions. The non-saturable permeability (P_d) in jejunum (44.6×10^{-4} cm/min) was approximately twice that in ileum (20.4×10^{-4} cm/min).
Conclusions. The permeability-index approach yielded parameters with reduced variability by eliminating potential imprecisions in length and surface area measurements of the intestinal segment perfused. D-glucose was transported via carrier-mediated systems in both jejunum and ileum, with different transport capacity and affinity in these two regions.

KEY WORDS: glucose; antipyrine; permeability; intestinal perfusion; rat.

INTRODUCTION

The intestinal perfusion technique has been used in studying various drug absorption characteristics including the mechanism of absorption, physicochemical or physiological factors governing absorption, and permeability differences at different intestinal regions. This technique has facilitated drug screening in the pharmaceutical industry because of its simplicity, efficiency and a good correlation between oral absorption and intestinal permeability in human or rat models (1–4). However, the variability of permeability measurements in general tends to be large, rendering it difficult to make quantitative comparisons between compounds. To compare results generated in different laboratories would be even harder because different experimental conditions and procedures are often used. Some standardization of intestinal perfusion procedures may be needed in order to allow proper interpretation and extrapolation of the permeability data. The present report describes a permeability-index approach, in which an internal standard is perfused together with the test compound. The ratio of the permeability of the test compound to that of the internal standard is defined as the permeability-index. It is hoped that by including an internal standard in the perfusate, the experimental variability in parameter estimates could be reduced.

D-glucose absorption from the intestine has been assumed to occur primarily via an active, carrier-mediated process (5,6) with the paracellular route in jejunum playing a minor role (~5%) (7,8). In the present study, the transport of D-glucose in rat jejunum and ileum was investigated over a wide concentration range. Antipyrine, known to be transported passively and has been used as a tissue viability marker in intestinal perfusion experiments (3), was selected as an internal standard.

MATERIALS AND METHODS

Drugs and Perfusate Solutions

The specific activities of [^{14}C]antipyrine and [^{3}H]D-glucose (Sigma Chemical Co. St. Louis, MO) were 14 mCi/mmol (74.4 μCi/mg) and 15.5 Ci/mmol (86.0 μCi/μg), respectively. MES (2-(N-morpholino)ethane-sulfonic acid) was purchased from Sigma Chemical Co. (St. Louis, MO). Glucose and antipyrine perfusate solutions were prepared in MES buffer (10 mM MES, 135 mM NaCl, 5 mM KCl, adjusted to pH 6.5 by 1N NaOH solution).

Surgery

A single-pass intestinal perfusion technique was used. Rats fasted overnight with water *ad libitum* were anesthetized with isofluorane inhalation. Surgery was performed as described previously for a rabbit model (9) with some modifications. Briefly, the rat was laid in a supine position on a heating pad to maintain body temperature throughout the experiment. Following induction of anesthesia, the abdominal area was shaved and cleaned with alcohol. After onset of deep anesthesia, laparotomy was performed. The desired intestinal regions (an approximately 15–20 cm portion of the upper jejunum or ileum) were identified and ligated with silk thread. The proximal end of the intestinal segment was cannulated and connected to a perfusion syringe on a variable speed compact infusion pump, and the distal end was cannulated for perfusate collection. The intestinal segment was cleaned by perfusing with 37°C normal saline. The remaining saline was cleared by infusing air. The intestinal region under study was carefully arranged in a uniform S to multi-S pattern to avoid kinks and ensure a consistent flow. Saline soaked, warmed, sterile cotton was used to cover

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opened body cavities to prevent loss of body temperature and fluids.

Intestinal Perfusion and Sample Collection

The drug solution was perfused into the two intestine segments simultaneously at a perfusion rate of 0.2 mL/min. Intestinal effluent samples were collected into pre-weighed glass vials every 10 min for up to 100 or 120 min. Each effluent fraction was re-weighed after collection. At the end of the experiment, the drug solution was expelled by infusing air and then flushing with normal saline through the intestinal segment. The segment was gently separated from the mesenteric tissue and removed from the body. The length of the segment was measured with a ruler and used for permeability or permeability-index calculation. The intestinal segments were then solubilized (Study 2 only) with 30% methanolic potassium hydroxide (1 mL/g sample weight) before analyzed for ^{3}H and ^{14}C radioactivity.

Experimental Design

Study 1

Two intestinal segments from the jejunum and ileum of the same rat (n = 4) were perfused simultaneously with a solution containing [^{3}H]D-glucose (0.46 ng/mL, 2.56 nM) and [^{14}C]antipyrine (0.44 μg/mL, 2.34 μM) for 120 min. ^{3}H- and ^{14}C-radioactivity concentrations in the effluents and perfusates were determined.

Study 2

Two segments from the jejunum and ileum of the same rat were perfused simultaneously with a solution containing the same concentrations of [^{14}C]antipyrine and [^{3}H]D-glucose as in Study 1, but also unlabeled D-glucose at a concentration of 1, 3, 10, 30, 50, 100, 150, or 300 mM. One rat was used for each perfusate solution.

Analysis of Radioactivity

The radioactivity of all samples was determined by liquid scintillation counting in a Packard Spectrometer. Effluent and perfusate samples (0.2 mL) were counted directly after mixing with 2 mL of Formula 989 Scintillant (Parkard, Dowers Grove, IL). The solubilized intestinal segment was mixed with a sufficient quantity of ethanol to yield a final volume four times greater than the volume of 30% methanolic potassium hydroxide solution used. Triplicates of the final material (125–150 mg) was counted directly after mixing with 10 mL of Hionic-Fluor (Parkard, Dowers Grove, IL).

Data Analysis

Permeability-Index

The fraction of drug not absorbed at steady state during perfusion was described by the following equation:

$$\frac{R_{out}}{R_{in}} = \exp\left(-\frac{P_e AL}{Q_{in}}\right) \quad (1)$$

where R_{out} and R_{in} are the rates of drug leaving and entering the intestinal segment perfused, respectively, and equal to the product of the volumetric flow rate and drug concentration, $Q_{out}*C_{out}$ and $Q_{in}*C_{in}$. Q_{out} and Q_{in} may differ due to water absorption or secretion during the perfusion. A is the effective surface area per unit length of the intestinal segment perfused (cm^2/cm), which in practice is often substituted by the anatomical surface area ($2\pi r$, where r is the anatomical radius of intestine assumed to be 0.2 cm). L is the anatomical length of the intestinal segment perfused. P_e is the effective permeability coefficient (cm/min), which can be obtained by rearranging Equation 1,

$$P_e = -\frac{Q_{in}}{AL}\ln\left(\frac{R_{out}}{R_{in}}\right) \quad (2)$$

When a study compound (c) and an internal standard (s) are perfused simultaneously, the ratio of the permeability of the study compound ($P_{e,c}$) versus that of the standard ($P_{e,s}$), henceforth referred to as the permeability-index (P_i), can be described using the following equation:

$$P_i = \frac{P_{e,c}}{P_{e,s}} = \ln\left(\frac{R_{out}}{R_{in}}\right)_c / \ln\left(\frac{R_{out}}{R_{in}}\right)_s \quad (3)$$

P_i depends only on the concentration and flow rate measurements. It makes no assumption about the anatomical nature of the surface area for absorption and is independent of the accuracy of intestinal segment length measurements. If the same internal standard is used in all perfusion experiments, a reliable population mean for the permeability of the internal standard, $P_{e,s}$, can be determined. $P_{e,c}$ is then calculated simply as $P_{e,s} \cdot P_i$.

In the present study, the transport of D-glucose was studied using the permeability-index approach with antipyrine as an internal standard. The permeability-index of D-glucose at varying concentrations in the perfusate can be described using the following equation assuming that D-glucose is transported via a carrier-mediated system as well as by passive diffusion:

$$P_i = \frac{V_{max}/P_{e,s}}{K_m + C_{per}} + P_d/P_{e,s} \quad (4)$$

where V_{max} is the maximum carrier flux (μmole/min/cm^2); K_m is the carrier dissociation constant (μmole/mL); C_{per} is the concentration of D-glucose in perfusate (μmole/mL). $P_{e,s}$ is the permeability of antipyrine (cm/min). P_d is the non-saturable permeability of D-glucose (cm/min), which represents the sum of: 1) paracellular passive diffusion, 2) transcellular passive diffusion, and 3) other transcellular carrier-mediated transport within the linear range. The model parameters, V_{max}, K_m and P_d, were estimated through nonlinear regression analysis using WinNonlin (Version 1), with $P_{e,s}$ fixed at the mean value from all study animals. For comparison purposes, parameter estimates were also obtained by fitting the equation $P_e = V_{max}/(K_m + C_{per}) + P_d$ to the data.

Tissue/Perfusate Distribution of Radioactivity

The distribution of ^{3}H- or ^{14}C-radioactivity between intestine tissue and perfusate was calculated based on the radioactiv-

ity concentrations in the homogenate of intestinal segment perfused and the mean perfusate concentration inside the lumen. The mean radioactivity concentration inside the lumen at steady state ($C_{per,mean}$, DPM/mL) was estimated using the following equation:

$$C_{per,\,mean} = \frac{C_{in} - C_{out}}{\ln\left(\frac{C_{in}}{C_{out}}\right)} \quad (5)$$

The radioactivity concentration in the intestinal segment perfused (DPM/g-tissue) was determined after solubilization. The relative distribution of ^3H versus ^{14}C between intestinal tissue and perfusate was also calculated.

Statistics

An one-tailed, paired t-test was used to test for significantly different results between jejunum and ileum. A value of $P < 0.05$ was considered significant.

RESULTS

With a perfusion rate of 0.2 mL/min, steady-state concentrations of antipyrine and glucose in effluents were achieved within one hour perfusion, and the effluent flow rate was mostly stabilized after the first two fractions. The earlier fractions may contain some saline solution left from cleaning of the intestinal lumen. In the present study, effluent flow rate (Q_{out}), permeability (P_e) and/or permeability-index (P_i) values were obtained based on measurements from the last four effluent fractions. The effluent flow rates at different glucose concentrations are plotted in Figure 1. Water absorption was observed when glucose concentration was ≤150 mM, and water secretion was observed at 300 mM in both jejunum and ileum. At glucose concentrations of ≤150 mM the percentage of net water flux, expressed as $(Q_{out} - Q_{in})/Q_{in} \times 100\%$, was $-(12 \pm 6.2)\%$ and $-(13 \pm 6.5)\%$ (11 rats) in jejunum and ileum, respectively. At 300 mM, net water flux was +7.5% and +18.5% (one rat), respectively.

The effective permeability coefficient and permeability-index values of D-glucose and antipyrine in jejunum and ileum from Study 1 are listed in Table 1. The variability (CV%) for the permeability of D-glucose and antipyrine was very high, 23–36%, but the variability for their ratio, referred to as the permeability-index of D-glucose, was only ~5%. The permeability of D-glucose in jejunum was higher than that in ileum, and the statistical significance of the difference was enhanced when the permeability-index values were compared, the p-value decreasing from 0.028 to 0.0004.

Table 1. Effective Permeability Coefficient (cm/min×10^4) and Permeability-Index of D-glucose and Antipyrine in Jejunum and Ileum

	Jejunum			Ileum		
Rat No.	$P_{e,glucose}$ [a]	$P_{e,antipyrine}$ [b]	P_i [c]	$P_{e,glucose}$ [a]	$P_{e,antipyrine}$ [b]	P_i [c]
1	152	106	1.43	89	79	1.12
2	147	99	1.48	107	93	1.15
3	257	169	1.52	158	129	1.23
4	120	74	1.63	106	86	1.23
Mean	169	112	1.52	115	97	1.18
SD	60	40	0.09	30	22	0.06
CV%	35.7	36.1	5.7	26.1	22.9	4.8

[a] Significantly different by one-tailed paired Student *t* test: p = 0.028.
[b] Not significantly different: p = 0.136.
[c] Significantly different: p = 0.0004.

The permeability of antipyrine in both jejunum and ileum was independent of the glucose concentration in the range tested, with the possible exception at the high end, 300 mM, where a below-average permeability was observed in the ileum (50.1×10^{-4} cm/min). The average permeability of antipyrine (including the 300 mM concentration) in jejunum and ileum were $(103 \pm 27.6) \times 10^{-4}$ cm/min and $(91.7 \pm 22.2) \times 10^{-4}$ cm/min, respectively, showing no significant difference between the two intestinal regions ($p > 0.05$). In contrast, the permeability of D-glucose was different between jejunum and ileum, the permeability-index of D-glucose in jejunum being consistently higher than that in ileum at each perfusate concentration tested (Figure 2). The permeability-index of D-glucose

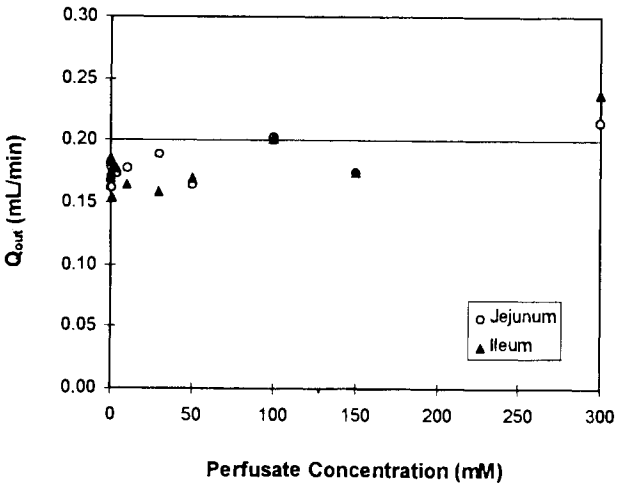

Fig. 1. Effluent flow rate vs. D-glucose concentrations in perfusate. Q_{in} was 0.2 mL/min.

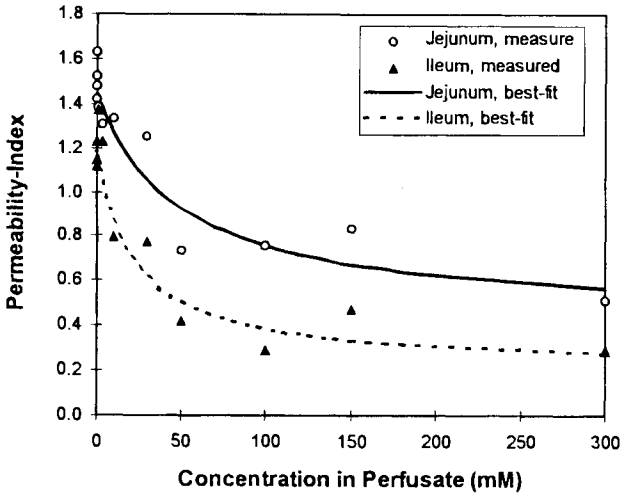

Fig. 2. Experimentally determined and best-fit permeability-index values of [^{3}H]D-glucose in jejunum and ileum of rats at different concentrations. [^{14}C]antipyrine was used as an internal standard. Results were from Studies 1 and 2.

Table 2. Transport Parameters of D-Glucose in Rat Jejunum and Ileum Calculated Using the Permeability-Index Approach as Compared with Using Permeability Directly

	Jejunum	%S.E.	Ileum	%S.E.
Using P_i Values[a]				
V_{max} (μmole/min/cm^2)	0.522	71	0.199	56
K_m (μmole/mL)	48.2	57	21.4	49
P_d (cm/min × 10^4)	44.6	42	20.4	51
V_{max}/K_m (mL/min/cm^2 × 10^4)	108	—	93.0	—
Using P_e Values				
V_{max} (μmole/min/cm^2)	0.263	153	0.361	124
K_m (μmole/mL)	21.4	133	33.4	103
P_d (cm/min × 10^4)	47.1	80	9.05	332
V_{max}/K_m (mL/min/cm^2 × 10^4)	123	—	108	—

[a] $P_{e,s}$ values of antipyrine were fixed at 103×10^{-4} cm/min in jejunum and 91.7×10^{-4} cm/min in ileum.

also showed concentration-dependency. The P_i value was similar between 2.56 nM and 1 mM, but decreased substantially as the perfusate concentration increased to 50 mM. The P_i value remained virtually constant between 50 mM and 300 mM. Results of nonlinear regression analysis are given in Table 2. Using the permeability-index approach (Equation 4) resulted in a considerably smaller relative standard error (%S.E.) for all three model parameters (V_{max}, K_m, and P_d) than using the permeability value directly, 42–71% vs. 80–332%. Fitting P_i or P_e data yielded comparable estimates of the maximum active transport efficiency (V_{max}/K_m), but markedly different values of the individual parameters (V_{max}, K_m, P_d).

The distribution of radioactivity between the intestinal segment perfused and the average concentration in perfusate at steady state was plotted in Figure 3. Antipyrine showed similar tissue distribution regardless of the presence of varying concentrations of D-glucose in both jejunum and ileum. The tissue-to-perfusate distribution ratios of [^{14}C]antipyrine in jejunum and ileum were not significantly different, 0.58 ± 0.09 and 0.54 ± 0.06, (n = 8), respectively. In contrast, the tissue/perfusate distribution of [^{3}H]D-glucose was concentration-dependent. The distribution-ratio in both perfused regions decreased substantially when D-glucose concentration increased from 1 to 50 mM, and remained virtually constant at concentrations between 50 mM and 300 mM. Tissue distribution or binding of D-glucose in ileum appeared to be greater than that in jejunum at all concentrations examined.

DISCUSSION

The variability in permeability estimates of a given compound may be attributed to any of the following factors: 1) different experimental conditions, e.g. different perfusate solutions, pH, osmolarity, temperature, and anesthesia (3); 2) inprecision of length measurement of the intestinal segments perfused (L); and 3) interanimal differences in terms of the radius of intestine or the actual surface area per cm for absorption (A).

Based on Equations 2 and 3, the accuracy in length (L) and area (A) measurements will affect the estimate of permeability, but not the proposed permeability-index. This hypothesis was supported by results in the present study, the variability in permeability-index of D-glucose being much smaller than that of the permeability of D-glucose or antipyrine alone. Indeed the experimental variability in L and A measurements could be large. Our experience showed that the anatomical length of intestinal segment removed from the body may vary by as much as 50% depending on whether or not the segment is moistured with saline, measured at 37°C or at room temperature, before or after perfusion, with or without perfusate in the lumen, and with or without connective tissues if the segment is measured after removed from the body. The anatomical radius of the intestine is also governed by these factors, with literature values ranging from 0.18 cm to 0.45 cm (1,3,10). Furthermore, since the anatomical surface area is smaller than the actual surface area (A) for transport, i.e. brush border membrane, by several orders of magnitude (11), any interanimal variability in the actual surface area will further contribute to the variability in permeability estimates.

A good internal standard in intestinal perfusion studies should meet several minimum criteria: 1) transported passively, 2) permeability unaffected by the test compound, and 3) does not alter intestinal physiology or the permeability of the test compound. In addition, good permeability and similar permeability value in the entire small intestine are preferred. It has been reported that antipyrine is transported passively from the small intestine and has a good intestinal permeability (3). The

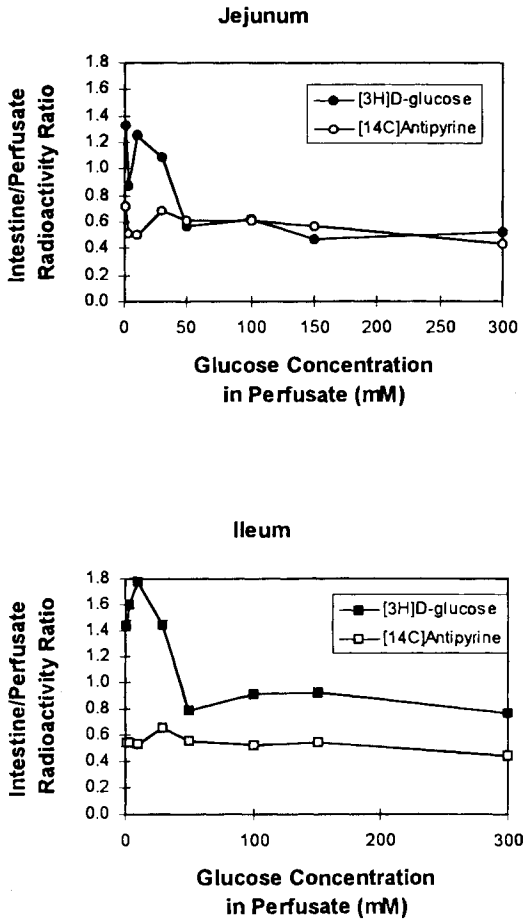

Fig. 3. Distribution of [^{3}H]D-glucose and [^{14}C]antipyrine in the homogenate of intestinal segment perfused and in the perfusate at steady state.

present study has further shown that antipyrine has similar permeability and tissue binding in the upper (jejunum) and lower (ileum) regions of the small intestine. The compound also experiences no intestinal metabolism (3). In proposing the use of antipyrine as a universal internal standard, it is assumed that the permeability of antipyrine is unaffected by the test compound. Although the conconmitant administration of other drugs could influence the GI absorption of antipyrine (12,13), there is no evidence that the effect is on the permeability of antipyrine. Conflicting results have been reported on the effect of intestinal blood flow on the GI absorption of antipyrine (14,15), thus warranting further investigation on the relationship between intestinal blood flow and antipyrine permeability.

In the present study, the transport of D-glucose was examined across a wide concentration range, from 2.56 nM to 300 mM. By using antipyrine as an internal standard (P_i approach), and thus removing measurement errors in intestinal radius and length, the resulting parameter estimates showed much smaller error than using P_e data directly (Table 2). Therefore results from the former analysis were judged to be more reliable. D-glucose was found to be transported via a carrier-mediated system and showed concentration dependency. The K_m value in jejunum was 48.2 μmole/mL, which was similar to that reported previously in the same species under similar experimental conditions (45 μmole/mL) (16). It has been reported that the K_m of the glucose transporter may be in the range of 2 to 5 mM when corrections are made for unstirred layers (16,17). Jejunum and ileum appeared to be different in the transport of D-glucose. The K_m and V_{max} in ileum were less than half of the respective values in the jejunum, rendering a comparable maxmum active transport efficiency (V_{max}/K_m) in jejunum and ileum. A higher K_m in jejunum (thus lower affinity to the transporter protein) was consistent with a lower tissue binding in jejunum than in ileum. The non-saturable permeability (P_d) in the jejunum was approximately two times that in ileum.

The non-saturable permeability (P_d) in both jejunum and ileum was significant compared with the total transport efficiency. In jejunum P_d accounted for 29% of the total transport efficiency ($P_d + V_m/K_m$) and, in ileum, 17.8%. These values were much greater than the contribution from paracellular pathway alone (~5% (7)). As described by Equation 4, P_d represents the sum of all non-saturable permeabilities. If the paracellular pathway was minimal and transcellular passive diffusion of D-glucose was negligible as for other L-sugars (7,8), the relatively large non-saturable permeability (P_d) observed in both intestinal regions might be attributed to one or more of the D-glucose transporters identified (18).

In conclusion, by introducing an internal standard in the perfusate, the permeability of D-dglucose was characterized with substantially reduced variability. The use of permeability-index eliminated the inprecision in measuring the length of the intestinal segment perfused and the actual surface area available for absorption. Antipyrine, transported passively with good permeability, appeared to be a suitable internal standard for intestinal perfusion studies. The transport of D-glucose in the small intestine was concentration-dependent and differed between the jejunum and ileum. The active transporter in jejunum has a larger capacity but lower affinity as compared with that in the ileum, but the maximum transport efficiency mediated by the carrier was similar in both regions. The non-saturable permeability was greater in the jejunum than that in the ileum, and both exceeded the reported paracellular permeability of D-glucose. It appears that the transport of D-glucose may be mediated by more than one D-glucose transporters in the small intestine.

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