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Comparative assessment of the resistance of the unstirred water layer to solute transport between two different intestinal perfusion systems

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The resistance of the unstirred water layer to solute transport was estimated in two different intestinal single-pass perfusion systems for a comparative study, using D-glucose as a model compound. One is a well established perfusion system in anesthetized rats as a standard (system A). The other is the one in unanesthetized rats for comparison (system B). It was demonstrated that in system B as well as in system A the resistance of the unstirred water layer to D-glucose transport should be taken into account and this resistance, accordingly, the effective thickness of the unstirred water layer (δ) which is assumed to be in proportion to its resistance, could be described as a function of the perfusion rate by using a film model. The δ decreased with increasing perfusion rate and was larger in system A than in system B at each perfusion rate; 785 μm in system A versus 319 μm in system B at the perfusion rate of 0.16 ml/min and 337 μm versus 184 μm at that of 2.95 ml/min. Thus in system B the effective thickness, accordingly, the resistance, of the unstirred water layer was reduced to about 50% of that in system A, but the resistance of the unstirred water layer could still account for 85% of the total resistance at the maximum as far as D-glucose absorption was concerned, while 93% in system A. These results suggest that, compared with perfusion experiments in anesthetized rats (system A), the resistance of the unstirred water layer is reduced but cannot be left out of consideration even if perfusion experiments are performed in unanesthetized rats (system B). And the lower resistance of the unstirred water layer in system B was attributed to a turbulent flow in contrary to a laminar flow in system A.

Introduction

The resistance of the unstirred water layer to solute transport is a well established concept which should be considered in interpreting the data of solute transport across biological membranes [1]. And it should also be considered in intestinal perfusion experiments.

In a particular perfusion system, a single-pass perfusion of the small intestine out of the abdomen of the anesthetized rat, the resistance of the unstirred water layer and its effects on the kinetic parameters for solute transport have been extensively investigated, because stabilized pseudo-cylindrical geometry can be easily maintained in this system and it is of advantage to quantitative studies. For operational purpose a film model has been traditionally used [2–4], which consists of the

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bulk phase without concentration gradient and the unstirred water layer adjacent to the mucosal surface with linear concentration gradient. In this model the resistance of the unstirred water layer to transport of a given solute is assumed to be in proportion to the effective thickness of the unstirred water layer and in inverse proportion to the diffusion constant of the solute. Komiya et al. demonstrated that the resistance, accordingly the effective thickness, of the unstirred water layer can be described as a function of the perfusion rate by using steroids as model compounds [5]. On the other hand it has recently been suggested that the assumption of laminar flow in a straight cylinder with smooth surface is applicable to this perfusion system as a major mechanism which can produce the resistance of the unstirred water layer [2,3,6–12]. However, in other perfusion systems only limited information is available as to quantitative estimation of the resistance of the unstirred water layer [13–16].

In order to quantitatively elucidate the intestinal absorption mechanism we must characterize various perfusion systems, including estimation of the resistance of the unstirred water layer. And then the resistance of the unstirred water layer in vivo and such factors peculiar to in vivo as intestinal motility will have to be assessed.

In this study we intended to estimate the resistance of the unstirred water layer in two different perfusion systems for a comparative study, in an attempt to characterize various perfusion systems. One is the above mentioned well established perfusion system in anesthetized rats as a standard. The other is the perfusion system in unanesthetized rats [13,14] for comparison. The latter was chosen because it is unique in the use of unanesthetized rats and the smaller resistance of the unstirred water layer than those in anesthetized rats is expected but the resistance was only estimated by the measurement of the change in the potential difference but not the solute transport. In this study D-glucose was used as a model compound and its nonlinear absorption kinetics was analyzed, using a film model and the approach of Komiya et al. [5] in the light of the relation between the absorption rate of the noticed solute, the resistance, or the effective thickness, of the unstirred water layer and the perfusion rate.

Theory

It is assumed that the intestinal tract is a straight cylinder with smooth surface and that the sink condition in the blood is maintained. The distance from the inlet along the intestinal tract is denoted by x. According to mass balance considerations, the difference between the rate of a given solute entering into a cylindrical element, a small segment of the intestinal cylinder, at x and that leaving at $x + \Delta x$ is equal to the transverse flux of the solute across the intestinal membrane within the element.

Model 1: a film model considering the unstirred water layer

In the presence of passive transport alone, equations derived by Ho and Higuchi [17] and Komiya et al. [5] are used. The equation considering the mass balance is as follows:

$$Q \cdot C(x) - Q \cdot C(x + \Delta x) = 2\pi R \cdot \Delta x \cdot P_{app} \cdot C(x) \quad (1)$$

where

$$\frac{1}{P_{app}} = \frac{1}{P_m} + \frac{1}{P_{aq}} \quad (2)$$

$$P_{aq} = k \cdot Q^n = D/(\delta/10000) \quad (3)$$

and where Q is the perfusion rate in ml/s; C is the bulk concentration or the average concentration at a cross section (mM); R is the operational radius of the intestinal cylinder (cm); P_{app} is the apparent membrane permeability coefficient (cm/s); P_m is the memrbane permeability coefficient (cm/s); P_{aq} (cm/s) is the permeability coefficient of the unstirred water layer which is assumed to be a function of the perfusion rate and also related to the diffusion constant, D (cm^2/s), of the solute and the effective thickness of the unstirred water layer, $\delta(\mu m)$; k is a constant descriptive of the aqueous diffusivity of the solute, kinematic viscosity and geometrical factors; and n is an exponent of which $0 < n < 1$. The reciprocals of P_{app}, P_m and P_{aq} represent the total (apparent) resistance, the resistance of the intestinal membrane and the resistance of the unstirred water layer, respectively. Accordingly, the total resistance is recognized as the sum of two resistances in series. Dividing Eqn. 1 through by $-Q \cdot \Delta x$,

and taking the limit as $\Delta x \to 0$ yields

$$\frac{dC}{dx} = -\frac{2\pi R}{Q} \cdot P_{app} \cdot C \tag{4}$$

Given the initial condition of $C = C_{in}$ at $x = 0$, and integrating from $x = 0$ to L, the solution of $C = C_{out}$ at $x = L$ becomes

$$C_{out} = C_{in} \cdot \exp(-2\pi R \cdot L \cdot P_{app}/Q) \tag{5}$$

where C_{in} and C_{out} are the concentration in the inflow solution and the outflow solution, respectively; L is the distance of the outlet from the inlet or the length of the perfused segment. The absorption rate (nmol/min per length of the perfused segment) is described as follows:

$$\text{absorption rate} = Q \cdot (C_{in} - C_{out}) \cdot 1000 \cdot 60 \tag{6}$$

It is also represented as follows:

$$\text{absorption rate} = Q \cdot C_{in} \cdot F_a \cdot 1000 \cdot 60 \tag{7}$$

where F_a is the fraction absorbed of $1 - C_{out}/C_{in}$. From Eqn. 5 the P_{app} is represented as follows:

$$P_{app} = -\frac{Q}{2\pi R \cdot L} \cdot \ln \frac{C_{out}}{C_{in}} = -\frac{Q}{2\pi R \cdot L} \cdot \ln(1 - F_a) \tag{8}$$

In the presence of the Michaelis-Menten type carrier-mediated transport with simultaneous passive transport, using the expression of the absorption rate derived by Winne [4], the differential equation corresponding to Eqn. 4 is as follows:

$$\frac{dC}{dx} = -\frac{2\pi R}{Q} \cdot P_{aq} \cdot \left(C + F - \left(F^2 + q \cdot K_m \cdot C\right)^{1/2}\right) \tag{9}$$

where

$$q = \frac{1}{1 + P_m/P_{aq}} \tag{10}$$

$$F = \tfrac{1}{2} q \cdot \left(\frac{K_m}{q} + \frac{V_{max}/1000}{P_{aq}} - C\right) \tag{11}$$

and where K_m and V_{max} are the Michaelis constant (mM) and the maximal transport velocity (nmol/s per cm^2), respectively. Given the initial condition of $C = C_{in}$ at $x = 0$, and numerically integrating Eqn. 9 by Runge-Kutta-Gill method, $C = C_{out}$ at $x = L$ is obtained. The absorption rate is described by Eqn. 6 or 7. At the lower C_{in} where the concentration at the intestinal membrane surface is much lower than K_m, the flux across the intestinal membrane (or the absorption rate) is related linearly to C (or C_{in}) and Eqn. 8 can be adapted and where

$$\frac{1}{P_{app}} = \frac{1}{P_m + V_{max}/K_m} + \frac{1}{P_{aq}} \tag{12}$$

When the diffusional rate across the unstirred water layer is the rate-determining factor, i.e., $P_{aq} \ll P_m$ or $P_{aq} \ll P_m + V_{max}/K_m$,

$$P_{app} = P_{aq} = k \cdot Q^n \tag{13}$$

and then

$$\log P_{app} = \log k + n \cdot \log Q \tag{14}$$

Thus log P_{app} is related linearly to log Q.

Model 2: a model without the unstirred water layer

The equation corresponding to Eqn. 5 is as follows:

$$C_{out} = C_{in} \cdot \exp(-2\pi R \cdot L \cdot P_m^*/Q) \tag{15}$$

where P_m^* is the membrane permeability coefficient determined by this model.

The equation corresponding to Eqn. 9 is as follows:

$$\frac{dC}{dx} = -\frac{2\pi R}{Q} \cdot \left(P_m^* + \frac{V_{max}^*}{K_m^* + C}\right) \cdot C \tag{16}$$

where K_m^* and V_{max}^* are the Michaelis constant and the maximal transport velocity determined by this model, respectively. This differential equation is solved numerically in the same way as Eqn. 9.

The absorption rate is described by Eqn. 6 or 7.

Methods

Materials. D-[U-^{14}C]Glucose (14.4 mCi/mmol), L-[1-^{14}C]glucose (47.0 mCi/mmol), [G-^{3}H]inulin (430.6 mCi/g), Protosol (tissue solubilizer) and Bio-Fluor (scintillation cocktail) were purchased from New England Nuclear (Boston, MA). D-Glucose (Tokyo Kasei Kogyo Co., Tokyo) and L-glu-

cose (Sigma Chemical Co., St. Louis, MO) were commercially obtained. All other reagents were commercially obtained and were of analytical quality.

Animals. Male Wistar rats weighing 300–350 g were fed regular chow and water ad libitum and were not fasted prior to experiments.

Preparations. In perfusion system A, the 10 cm jejunal segment was perfused on a flat plate out of the abdomen of the anesthetized rat, maintaining stabilized pseudo-cylindrical geometry of the intestinal tract, as described in detail in our previous report [11]. Briefly speaking, the rat was anesthetized with urethane (4.5 ml/kg intraperitoneally, 25% solution). Cannulated with polyethylene tubing were the trachea, the left carotid artery, the right jugular vein, the perfused jejunal segment and the superior mesenteric vein draining it. Injected into the jugular vein were heparin (1 000 unit/ml, 0.22 ml) and phentolamine (2.5 mg/ml, 0.2 ml).

In perfusion system B, the 10 cm jejunal segment was perfused, as it was, in the abdomen of the unanesthetized rat in a Bollman cage. Under light ether anesthesia the abdomen of the rat was opened. The inflow cannula made of polyethylene tubing and the outflow cannula made of silicon tubing (internal diameter 0.3 cm) were attached to the 10 cm segment of the jejunum and its lumen was flushed with saline. Then the abdomen was sutured and the rat was put into a Bollman cage. Immediately after the rat awoke from anesthesia, perfusion of the jejunal segment was begun.

In both systems the jejunal segment was perfused by a single-pass method and by means of a peristaltic pump (Minipuls II, Gilson CO.). After 20 min of lag time the outflow solution, in both systems, and the blood from the mesenteric vein, only in system A, were collected at 5-min intervals for 20 min.

Solutions. Krebs-Ringer bicarbonate buffer (pH 7.4) containing a known concentration of unlabeled D-glucose with tracer amount of ^{14}C-labeled D-glucose and ^{3}H-labeled inulin as the marker for the intraluminal volume change was used as the perfusion solution. The osmolality of the perfusion solution was maintained to be 425 mosmol/kg by changing NaCl content in order to prevent the intraluminal volume change.

Analyses. The 10 ml scintillation cocktail (Bio-Fluor) was added to the 100-μl aliquots of the inflow solution and the outflow solution and the radioactivity was determined by liquid scintillation counting.

The 50-μl aliquots of blood were solubilized in 0.5 ml of a 2 : 1 mixture of ethanol and protosol (60 °C, 1 h) and decolorized with 0.3 ml 30% hydrogen peroxide (60 °C, 0.5 h); 10 ml scintillation cocktail and 0.5 ml 0.5 M HCl were added, and the radioactivity was determined.

Representation and treatment of data. The absorption rate (nmol/min per 10 cm) as the average of four sampling periods was estimated from disappearance of D-glucose from the lumen, using Eqn. 6, after correcting for the intraluminal volume change, though it was in the negligible level. This disappearance rate corresponded to the appearance rate into the mesenteric venous blood in every observation in system A, only in which the mesenteric venous blood could be collected. The operational radius of the intestinal cylinder (R) was estimated from the measurement of the outer circumference by $R = 0.83 \cdot (\text{outer circumference})/(2\pi)$, where the value of 0.83 represents the ratio of the inner to the outer circumference [11]. The R value of 0.234 cm estimated in system A at the perfusion rate of 0.16 ml/min was used in model analysis, as no significant change in the operational radius was observed when the perfusion rate was varied between 0.16 and 2.95 ml/min. It was also reported by Komiya et al. [5] that the radius and the intraluminal pressure did not change within the range of perfusion rate from 0.25 ml/min to 4.2 ml/min. The operational radius in system B was assumed to be equal to that in system A, though direct measurements could not be performed and the bias from the cylindrical geometry may be greater. The diffusion constant (D) of D-glucose is $7.04 \cdot 10^{-6}$ cm^2/s [11].

Kinetic parameters were obtained by fitting model 1, considering the unstirred water layer, to experimental data. Namely, Eqn. 5 in combination with Eqn. 6 and the numerical solution of Eqn. 9 in combination with Eqn. 6 were simultaneously fitted to data of L-glucose and D-glucose, respectively, using a nonlinear regression method, MULTI [18]. For comparison model 2, without unstirred water layer, was also fitted to the experi-

mental data in the same way, using Eqns. 6, 15 and 16.

Results

The absorption rate of D-glucose as a function of the concentration in inflow solution was examined at the perfusion rate of 0.16 ml/min (Fig. 1). The absorption rate showed concentration-dependent saturation kinetics in both perfusion systems, indicating the presence of carrier-mediated transport, though the absorption rate was larger in system B than in system A at every concentration.

The absorption rate of D-glucose as a function of the perfusion rate was examined at the concentration in inflow solution of 1 mM (Table I). In both perfusion systems the absorption rate increased as the perfusion rate increased.

The data shown in Table I were transformed into the relation between the apparent membrane permeability coefficient (P_{app}), which was estimated by using Eqns. 7 and 8, and the perfusion rate in ml/s, Q (Fig. 2). As the absorption rate was related linearly to C_{in} over a range of concentrations lower than 20 mM in both perfusion systems as shown in Fig. 1, Eqn. 12 is applicable. The linear relationship between log P_{app} and log Q suggests that in both perfusion systems the diffusional rate across the unstirred water layer is the rate-determining factor and Eqn. 13 is applicable as an approximation. If the resistance of the unstirred water layer were negligible or absence, namely, $1/P_{aq}$ in Eqn. 12 could be canceled or model 2 could be applied, P_{app} would not depend on the perfusion rate and it would be a constant. However, that is not the case. Here, initial estimates of n and k were obtained by a linear regression analysis and used in the following fittings, using equations without approximations.

The absorption rate of L-glucose, which was used to estimate the passive transport component of D-glucose absorption, was examined as a function of the concentration in inflow solution at the perfusion rate of 0.16 ml/min (Table II). The absorption rate showed concentration-independent linear kinetics and it was much smaller than that of D-glucose in each perfusion system, indi-

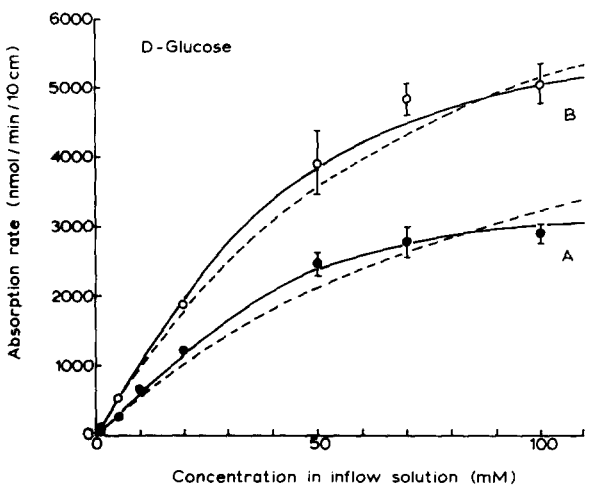

Fig. 1. Absorption rate of D-glucose as a function of concentration in inflow solution in the single-pass perfusion of the jejunum out of the abdomen of the anesthetized rat (system A) and in the abdomen of the unanesthetized rat (system B). The solid and dotted curves were obtained by fitting model 1 and model 2, respectively, to the experimental data as described in the text. The resistance of the unstirred water layer is taken into account in the former but not in the latter. Each point and vertical bar represents the mean ± S.E. of six experiments for $C_{in} = 1$ mM in both perfusion systems and $C_{in} = 100$ mM in perfusion system B and three experiments for the others.

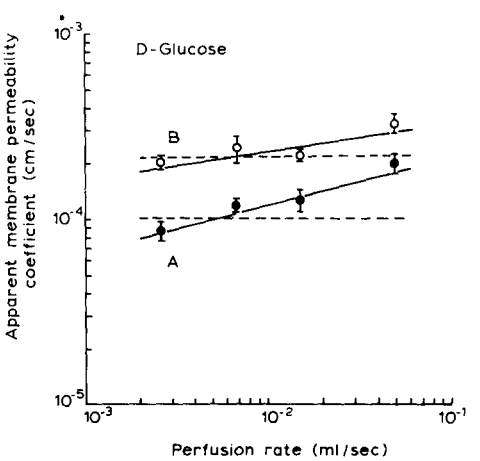

Fig. 2. Apparent membrane permeability coefficient versus perfusion rate in ml/s in system A and system B. Values of the apparent membrane permeability coefficient were obtained from the experimental data in Table I, using Eqns. 7 and 8. The solid and dotted curves were obtained by fitting model 1 and model 2, respectively, to the experimental data as described in the text. Each point and vertical bar represents the mean ± S.E. of six experiments for the lowest perfusion rate and three experiments for the others.

TABLE I

ABSORPTION RATE OF D-GLUCOSE AS A FUNCTION OF PERFUSION RATE

Values of the absorption rate represent the mean ± S.E. of six experiments for the perfusion rate of 0.16 ml/min in both perfusion systems and three experiments for the others. Values of the calculated absorption rate were obtained by fitting model 1 or model 2 to the experimental data as described in the text. C_{in} represents the concentration in the inflow solution.

Perfusion system	C_{in} (mM)	Perfusion rate (ml/min)	Absorption rate (nmol/min per 10 cm)	Calculated absorption rate (nmol/min per 10 cm)	
				Model 1	Model 2
A	1	0.16	59 ± 5	59	68
		0.40	92 ± 6	84	79
		0.90	102 ± 11	108	84
		2.95	169 ± 20	151	87
B	1	0.16	104 ± 5	102	114
		0.40	162 ± 19	151	159
		0.90	175 ± 10	190	180
		2.95	274 ± 32	241	194

cating the lack of carrier-mediated transport, though it was larger in system B than in system A in the same way as that of D-glucose.

Kinetic parameters for D-glucose absorption obtained in the model analysis are listed in Table III and calculated values or simulated curves are shown in Tables I and II and Figs. 1 and 2. Model 1 fitted better to the experimental data than model 2 in both perfusion systems. The AIC (Akaike's information criterion) values were smaller in model 1 than in model 2; 15.7 versus 38.9 in system A and 2.3 versus 7.7 in system B. The smaller AIC value indicates statistically better fit of the model [19,20]. The values of the Michaelis constant obtained by model 1 were almost the same in both systems, 3.0 ± 1.0 mM in system A and 4.8 ± 1.5 mM in system B, and corresponded to that of 3.3 mM obtained in our previous study using everted sacs [21], whereas those obtained by model 2 are one-order larger and different between system A and system B; 50.0 ± 1.8 mM versus 28.8 ± 4.0 mM. These larger values of the Michaelis constant obtained by model 2 are considered to be overestimates of the Michaelis constant due to the effects of the unstirred water layer [10,11]. The better fit of the model to the experimental data, the smaller AIC values and the consistency of the Michaelis constant among various experimental

TABLE II

ABSORPTION RATE OF L-GLUCOSE AS A FUNCTION OF CONCENTRATION IN INFLOW SOLUTION

Values of the absorption rate represent the mean ± S.E. of three experiments. Values of the calculated absorption rate were obtained by fitting model 1 or model 2 to the experimental data as described in the text. C_{in} represents the concentration in the inflow solution.

Perfusion system	C_{in} (mM)	Perfusion rate (ml/min)	Absorption rate (nmol/min per 10 cm)	Calculated absorption rate (nmol/min per 10 cm)	
				Model 1	Model 2
A	20	0.16	118 ± 25	99	99
	50		245 ± 31	247	247
	100		606 ± 86	493	494
B	1	0.16	11 ± 1	11	11
	20		274 ± 61	217	217

TABLE III

KINETIC PARAMETERS FOR D-GLUCOSE ABSORPTION OBTAINED IN THE MODEL ANALYSIS

Values are parameter ± S.D. which were obtained by fitting model 1 or model 2 to the experimental data as described in the text. Numbers of experiments are 42 and 39, respectively, for system A and system B.

Model	Perfusion system	Parameters					AIC [c]
		Michaelis constant (mM)	Maximal transport velocity (nmol/s per cm^2)	Membrane permeability coefficient (10^{-6} cm/s)	k ($\times 10^4$)	n	
1	A	3.0 ± 1.0	3.3 ± 0.3	6.1 ± 0.4	5.0 ± 1.1	0.29 ± 0.04	15.7
	B	4.8 ± 1.5	5.3 ± 0.3	13.5 ± 1.0	6.8 ± 2.1	0.19 ± 0.06	2.3
2	A	50.0 ± 1.8	4.8 ± 0.3	5.7 ± 0.5			38.9
	B	28.8 ± 4.0	6.4 ± 0.6	12.7 ± 0.9			7.7
Laminar flow [a]	A	4.5	3.4	7.0			
In vitro everted sacs [b]		3.3	2.3	10.7			

[a] Parameters were obtained in our previous study, using a model assuming two-dimensional laminar flow in the intestinal tract [11].
[b] Parameters were obtained in our previous study, using everted sacs [21].
[c] The smaller AIC (Akaike's information criterion) value indicates statistically better fit of the model [19,20].

systems support model 1 than model 2. Therefore, the resistance of the unstirred water layer should be taken into account in both perfusion systems and model 1 is appropriate in this context.

Discussion

This study was undertaken to assess the resistance of the unstirred water layer in the perfusion system in unanesthetized rats (system B) in comparison with the well established, as to the resistance of the unstirred water layer, perfusion system in anesthetized rats (system A). It was shown in the results section that in system B as well as in system A the resistance of the unstirred water layer should be taken into account and it can be appropriately described as a function of the perfusion rate by using a film model and the approach of Komiya et al. (model 1).

When the absorption rate shows concentration-dependent saturation kinetics, the contribution of the resistance of the unstirred water layer decreases as the concentration increases. However, at the linear portion of the absorption rate versus concentration in inflow solution curve we can assess it in the same way as the concentration-independent linear kinetics, using Eqn. 12. Values of the resistance of the unstirred water layer, $1/P_{aq}$, to D-glucose transport at the perfusion rate of 0.16 ml/min are $1.12 \cdot 10^4$ s/cm and $0.45 \cdot 10^4$ s/cm, respectively, in system A and system B, from $P_{aq} = k \cdot Q^n$ (Eqn. 3). Corresponding values of the total (apparent) resistance, $1/P_{app}$, at $C_{in} = 1$ mM are $1.20 \cdot 10^4$ s/cm and $0.53 \cdot 10^4$ s/cm, respectively, from Eqn. 8 and calculated values in the fittings. Accordingly, the resistance of the unstirred water layer can account for 93% of the total resistance in system A and 85% in system B. When the perfusion rate is increased to 2.95 ml/min, values of $1/P_{aq}$ are reduced to $0.48 \cdot 10^4$ s/cm and $0.26 \cdot 10^4$ s/cm, respectively, in perfusion system A and in system B but can still account for 84% and 74% of the total resistance, respectively, at $C_{in} = 1$ mM, because values of $1/P_{app}$ are also reduced to $0.57 \cdot 10^4$ s/cm and $0.35 \cdot 10^4$ s/cm, respectively. Therefore, at $C_{in} = 1$ mM and within the range of the perfusion rate used in this study the resistance of the unstirred water layer can account for most of the total resistance in both perfusion systems and the reduction in the resistance of the unstirred water layer is responsible for the increase in the apparent membrane permeability coefficient, P_{app}, with increasing perfusion rate (Fig. 2), in agree-

ment with the initial consideration as to the relation between P_{app} and the perfusion rate. And at the lower C_{in}, or the linear portion, as well as at $C_{in} = 1$ mM the larger absorption rate of D-glucose in system B than in system A (Fig. 1) is also accounted for by the smaller resistance of the unstirred water layer in the former. On the other hand values of the total resistance to L-glucose transport at the perfusion rate of 0.16 ml/min are $17.61 \cdot 10^4$ s/cm and $7.84 \cdot 10^4$ s/cm, respectively, in system A and system B. Accordingly, the resistance of the unstirred water layer can account for only 6% of the total resistance in both. Namely, the absorption rate of L-glucose is mostly determined by the diffusional rate across the intestinal memrbane and the smaller resistance of the intestinal memrbane, i.e., the larger membrane permeability coefficient (P_m), in system B than in system A is responsible for the larger absorption rate of L-glucose in the former (Table II). When carrier-mediated transport is saturated by the increase in the concentration, achieved maximal absorption rate must not be affected by the presence of the resistance of the unstirred water layer [10]. Because the maximal absorption rate is achieved by the presence of the sufficiently higher concentration at the intestinal membrane surface than the Michaelis constant regardless of the difference in the concentration, due to the unstirred water layer, between the intestinal membrane surface and the inlet or the bulk. Then, so far as the contribution of the resistance of the unstirred water layer to simultaneous passive transport component is negligible as D-glucose absorption in this study, the absorption rate at the saturated portion of the absorption rate versus concentration in inflow solution curve is little affected by the resistance of the unstirred water layer. Therefore, at the saturated portion the larger absorption rate of D-glucose in system B than in system A (Fig. 1) cannot be accounted for by the difference in the resistance of the unstirred water layer. It is mostly accounted for by the difference in the maximal transport velocity. Of course a passive transport component is also approximately 2-fold larger in system B than in system A as indicated by the absorption rate of L-glucose, or P_m, but its contribution to the absorption rate of D-glucose is small in each perfusion system; 16% in system A and 22% in system B even at the highest C_{in} of 100 mM.

The fact that the n value is larger in system A than in system B reflects a more conspicuous dependency of P_{app} on the perfusion rate in system A than in system B as shown in Fig. 2. The n value of 0.29 ± 0.04 in system A is smaller than that of 0.44 reported by Komiya et al. but corresponds to that of 0.333 expected for laminar flow in a straight cylinder with smooth surface [5]. The values of K_m, V_{max} and P_m in system A correspond to those obtained by a laminar flow model in our previous study [11]. These results support that laminar flow in a straight cylinder is a plausible mechanism which can produce the resistance of the unstirred water layer in system A. However, the assumption of laminar flow can not be applied to system B. Because values of the fraction of D-glucose absorbed, which is related to the absorption rate by Eqn. 7, were larger than the maximal value of 0.4 predicted by the laminar flow assumption [11] for the infinite membrane permeability, except for that at $C_{in} = 100$ mM. Especially they were 0.683 ± 0.026 and 0.717 ± 0.030, respectively, at $C_{in} = 1$ mM and 5 mM. These larger fractions absorbed cannot be explained by the assumption of laminar flow. And the n value of 0.19 ± 0.06 is smaller than 0.333 which is expected for laminar flow. It seems that in system B the intraluminal fluid flow is turbulent due to the bended small intestine in the abdomen and the less suppressed intestinal motility by the use of unanesthetized rats, compared with system A in which stabilized cylindrical geometry can be easily maintained on a flat plate out of the abdomen of the anesthetized rat. The turbulent flow can reduce the effective thickness, accordingly the resistance, of the unstirred water layer by producing a well-mixed bulk phase, compared with the laminar flow with no well-mixed bulk phase. Therefore, the observed lower resistance of the unstirred water layer in system B is attributable to the turbulent flow in contrary to the laminar flow in system A.

The effective thickness of the unstirred water layer (δ) can be estimated by Eqn. 3 as follows:

$$\delta = 10000 \cdot D/(k \cdot Q^n) \tag{17}$$

Here, the δ is presented for estimation of the

resistance of the unstirred water layer to various solutes by the relation of $1/P_{aq} = (\delta/10000)/D$ from Eqn. 3. The δ decreases as the perfusion rate increases and is larger in system A than in system B at each perfusion rate. For example, values of δ in system A and system B are 785 μm and 319 μm, respectively, at the perfusion rate of 0.16 ml/min, and 337 μm and 184 μm, respectively, at the perfusion rate of 2.95 ml/min. The δ in system B is about 50% smaller than that in system A, though, strictly speaking, this percentage depends on the perfusion rate. This percentage decrement represents the extent of the effect of the turbulent flow, assuming that δ in system A represents that in the laminar flow. In a limiting case of without unstirred water layer it becomes 100%. The resistance of the unstirred water layer to any given solute changes in proportion to δ. In system A comparative values of δ were reported by others [2–5,12]. In system B values of δ estimated by measuring the change in the osmotically induced potential difference by Hollander et al. are compatible with those in this study but less dependent on the perfusion rate; within the range from 200 μm to 300 μm when the perfusion rate was changed from 7.2 ml/min to 0.5 ml/min [13,14].

The value of V_{max} was 1.6-fold larger and that of P_m was 2.2-fold larger in system B than in system A. These may suggest the larger effective absorption surface area in the former than the latter, because P_m and V_{max} are parameters normalized by the same cylindrical surface area, an operational surface area, in both perfusion systems, neglecting the microgeometry due to the villous structure. They can reflect not only the pure permeability characteristics of the intestinal membrane but also the difference in the microgeometry of the intestinal membrane surface. Although the influence of villous structure on absorption has been treated theoretically by Winne [22], informations about the accurate geometry of the intestinal surface and factors affecting it are not available.

It should also be noticed that even the intestinal absorption in the perfusion experiments in unanesthetized rats may not correctly represent that in in vivo. Because in perfusion experiments the intestinal motility is probably suppressed by surgery and the luminal volume and the flow rate are far from physiological ones.

In conclusion the resistance, or the effective thickness, of the unstirred water layer is smaller in the perfusion system in unanesthetized rats (system B) than in the one in anesthetized rats (system A) but cannot be left out of consideration even in the former. It seems that the intraluminal fluid flow is turbulent in system B, while the laminar flow assumption is applicable to system A. And the lower resistance of the unstirred water layer in system B is attributable to a turbulent flow in contrary to a laminar flow in system A. The larger effective absorption surface area in system B than in system A was also suggested. Macroscopically the intestinal tract can be modeled as an cylindrical absorptive organ with a constant operational radius and the resistance of the unstirred water layer can be estimated as in this study, but microscopic consideration as to the geometry of the intestinal membrane surface and the intraluminal fluid flow will be needed to further elucidate the intestinal absorption mechanism.

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