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Influence of Perfusion Rate on the Kinetics of Intestinal Sugar Absorption in Rats and Hamsters *in vivo*

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The effect of perfusion rate (PR) on the apparent glucose and galactose-influx kinetics of rat and hamster jejunum *in vivo* has been studied. Total absorption (V) and absorption in the presence of 0.5 mM phloridzin (V_D) were measured in consecutive periods of 1 minute, and their difference ($V_T = V - V_D$) was taken as the mediated transport rate. PR values were 2.8, 5,6, 13.5 or 18.5 ml·min^{-1}, and the sugar concentrations in the perfusion solution (S_o) were 2, 5 and 10 mM.

Plots of $1/V_T$ versus $1/S_o$ for the different PR intercept the ordinate axis at the same point, yielding a common V_{max} for the same animal species and sugar. From the slopes, apparent K'_m values are obtained and apparent mass-transfer coefficients (K'_D) for the diffusion component are calculated (V_D/S_o) as well. When the PR increases, K'_n decreases, while K'_D increases.

A simplified model based on the assumption of a steady-state in which diffusion across unstirred water layers (UWL) equals the sum of a passive and a carrier mediated non-passive transepithelial transfer, acceptably accounts for the results. When taking as «true» K_m values the lowest ones reported in the literature, it is possible to obtain the sugar concentration at the enterocyte membrane (S_m), the effective UWL thickness (δ) and the «true» mass-transfer coefficient (K_D). S_o/S_m ratios and δ values decrease as PR increases, accounting for the biased K'_m and K'_D values. As it was expected, K_D did not change significantly by modifying PR.

Depending on sugar concentration, the passive component is almost equal to or much higher than the carrier-mediated transport, in rat as well as in hamster. Diffusion across unstirred water layers seems to be rate-limiting for intestinal sugar absorption.

Transfer of sugars from intestinal lumen to the subepithelial space requires the diffusion of the substrate molecules across the unstirred water layers (UWL) between the bulk phase and the absorbing cell surface (13, 14, 16, 26, 37, 38, 47-51) and then the crossing through the epithelium by two mechanisms, a non-passive carrier-mediated transcellular transport and a carrier-independent passive permeation

through a paracellular or cellular way (5, 10, 11, 19, 22, 45, 53). Experimental conditions, especially when *in vivo* techniques are used, influence the comparative importance of these intervening processes and thereby the kinetics of intestinal absorption. Unstirred layers act as a resistance that can be rate-limiting in the overall process of absorption (13, 35, 44, 50) and significantly bias the kinetic parameters for the different fluxes when not taken into consideration.

Previous experiments in this laboratory with evaluation of both the passive and non passive components (22) yielded apparent Michaelis constants for sugar carrier-mediated transport far less from those deduced from *in vitro* experiments. Furthermore an increase in sugar absorption with higher perfusion rates, was observed as it had been reported and imputed to several factors especially to the decrease in UWL thickness (9, 15, 22, 23, 29, 33, 35, 39, 54).

In the present paper, the influence of perfusion flow rate on the kinetics of glucose and galactose absorption across the rat and hamster jejunum has been studied *in vivo*. A simplified model has been adopted to follow absorption kinetics, which allowed an acceptable evaluation for the diffusion rate across the UWL, the passive and non-passive transepithelial fluxes, as well as for the sugar concentration at the aqueous membrane interfase and the effective thickness of the unstirred-layers.

Materials and Methods

Male and female Wistar rats (150-250 g) and golden hamters (90-105 g) were fasted for 24 h before the experiments. The preparation for perfusion *in vivo* has been described in detail elsewhere (33). Animals were anaesthetized with urethane (125 mg/100 g i.p.). A segment of proximal jejunum of about 15-20 cm length was cannulated and *in situ* perfusion was driven by a Harvard Peristaltic Pump. Flow rates were 2.8, 5.6, 13.5 or 18.5 $ml \cdot min^{-1}$. Single pass perfusion was used in all experiments. The sugars to be absorbed were added at 2, 5 or 10 $mmol \cdot l^{-1}$ to Krebs-Ringer-Tris (pH 7.4) solution (28, 46). Bubbles in the perfusion circuit were not allowed.

In the same jejunal segment, absorption rate was measured along 9-12 consecutive periods of 1 minute. The lumen was thoroughly washed before and after each absorption period with isotonic saline solution. The order for the experimental conditions in the periods was randomized in the different animals.

Absorption was estimated as the difference between the sugar contents in the inflow and outflow solutions. The concentration difference was sufficient to give reliable values for the disappearance rate of the sugar from the jejunal lumen. However, as it was usually lower than 10 %, the influence of the concentration decrease along the intestine on the absorption rate was not taken into consideration.

D-glucose and D-galactose were determined by the NELSON-SOMOGYI method (32, 42). Absorption rate is expressed in nanomoles per minute per centimeter of jejunum length (33). Under the adopted experimental conditions, volume changes in the perfusate from water net flux were always lower than 1 % and have therefore been neglected.

Total inhibition of the carrier-mediated transport was obtained by adding 0.5 mM phloridzin (1-4, 10, 11, 22, 34), the remaining absorption being accounted for by passive flux.

In some experiments, the absorption of a given sugar at different concentrations with or without phloridzin was compaired in the same jejunal segment at equal perfusion rate. In other animals the sugar concentration was kept the same, while the perfusion rate changed in the different consecutive periods.

Table I. *Influence of the perfusion rate (PR, ml·min^{-1}) on the intestinal absorption of D-glucose and D-galactose by rat and hamster jejunum* in vivo.
S_o = sugar concentration (mM) in the perfusion solution. Absorption rate in the absence (V) and in the presence (V_D) of 5×10^{-4} M phloridzin, in nmoles·cm^{-2}·min^{-1}. Mediated transport rate, $V_T = V - V_D$. Each value is the mean of 4 to 10 data. The S.E. of the mean was always 4 %.

S_o	PR:	2.8	5.6	13.5	18.5	2.8	5.6	13.5
		D-GLUCOSE			RATS	D-GALACTOSE		
2	V	61	78	100	119	62	86	101
	V_D	32	40	50	59	31	48	52
	V_T	29	38	50	60	31	38	49
5	V	137	186	228	285	137	201	246
	V_D	72	107	133	154	71	115	138
	V_T	65	79	95	131	66	86	108
10	V	237	325	395	486	261	342	411
	V_D	140	200	240	305	141	208	247
	V_T	97	125	155	181	120	134	164
					HAMSTERS			
2	V	62	—	93	—	62	—	95
	V_D	31	—	47	—	31	—	48
	V_T	31	—	46	—	31	—	47
5	V	140	—	240	—	142	—	215
	V_D	69	—	140	—	70	—	115
	V_T	71	—	100	—	72	—	100
10	V	249	—	395	—	268	—	415
	V_D	137	—	253	—	140	—	233
	V_T	112	—	142	—	128	—	182

Results

Absorption rates of D-glucose and D-galactose both in the absence (V) and presence of phloridzin (V_D), at different sugar concentrations and perfusion rates for rat and hamster jejunum are shown in table I.

As it may be seen, in both animal species when the perfusion rate increases, V and V_D values for each concentration of sugar also increase.

If the rates of the non passive transport, V_T, measured as $V-V_D$, were plotted against the sugar concentrations in the solution, S_o, saturation curves could be obtained for each perfusion rate (PR), which acceptably fitted MICHAELIS-MENTEN kinetics, making possible to calculate the corresponding values for the apparent semisaturation constant, K'_m, and the maximal saturation rate V'_{max}, from the LINEWEAVER-BURK double reciprocal plots (figs. 1, 2 and table II).

As the figures, show the reciprocal straight lines. for the same species and sugar at different perfusion rates, intercept ordinate axis at the same point, giving a common value for V_{max}. However, the K'_m values decrease as perfusion rate increases.

The variation in K'_m as a function of perfusion rate has to be mostly attributed to changes in the resistance of the unstirred water layers to sugar diffusion (6, 16, 17, 23, 24, 31, 35, 37, 40, 43, 45, 48, 51-54).

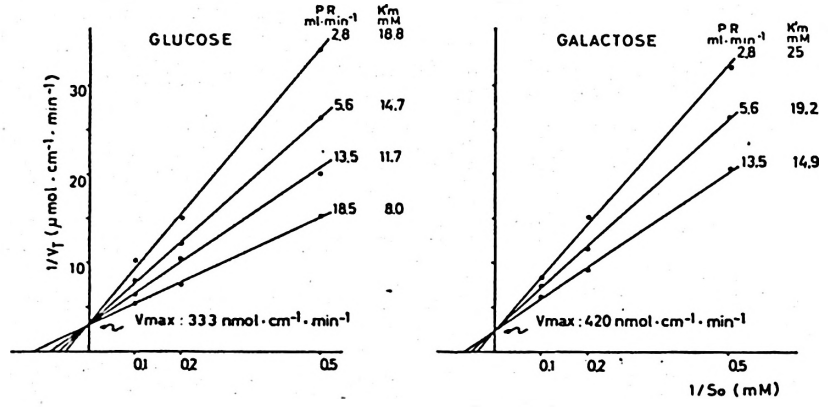

Fig. 1. *Lineweaver-Burk plots for glucose and galactose transports in the rat, at different perfusion rates.*

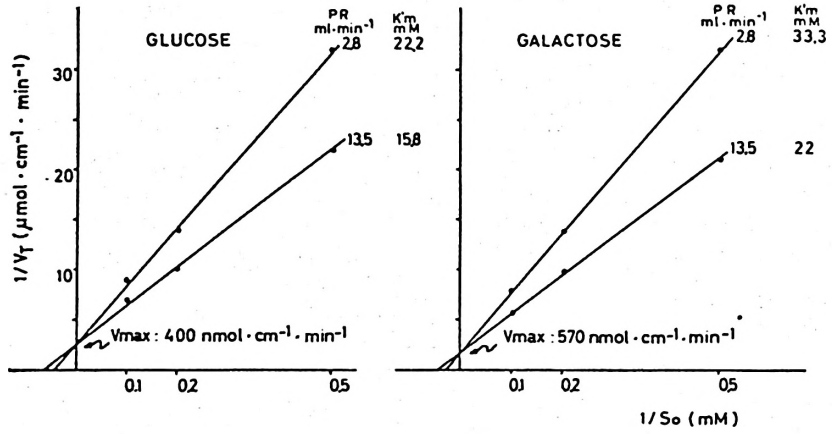

Fig. 2. *Lineweaver-Burk plots for glucose and galactose transports in the hamster, at different perfusion rates.*

and therefore to changes in the effective concentration of sugar at the aqueous-membrane interface, S_m.

Under the assumption of a negligible concentration of sugar in subepithelial space, the values of passive diffusion component across the epithelium, V_D, is described by the equation for each perfusion rate:

$$V_D = K'_D \cdot S_o$$

where K'_D is an apparent mass-transfer coefficient. These values of K'_D for the different perfusion rates, animals and sugars are also given in table II.

Table I shows the great relevance of the passive component V_D, that amounts to as much as the carrier-mediated transport at 2 mM sugars, and still much more at higher sugar concentrations.

ADOPTED THEORETICAL MODEL. That sugar disappeared from the solution as the fluid moved along the jejunum segment may be accounted as a result of

Table II. *Apparent kinetic constants for sugar absorption by rat and hamster jejunum in vivo, at different perfusion rates (PR, ml·min^{-1}). K'$_m$ (mM) and V$_{max}$ (nmoles·cm^{-1}·min^{-1}) for the mediated transport component.*
K'$_D$ (nmoles·cm^{-1}·min^{-1}·mM^{-1}) for the diffusion component.

PR:	2.8	5.6	13.5	18.5	2.8	5.6	13.5
		D-GLUCOSE		RATS	D-GALACTOSE		
K'$_m$	18.8	14.7	11.76	8.0	25	19.23	14.9
V$_{max}$	333				420		
K'$_D$	14.8 ± 0.8	20.4 ± 0.6	25.2 ± 1.0	30.2 ± 0.5	14.6 ± 0.6	22.6 ± 1.3	26.1 ± 1.2
				HAMSTERS			
K'$_m$	22.2		15.8		33.3		22.0
V$_{max}$	400				570		
K'$_D$	14.3		25.6		14.5		23.4

two in series processes: 1) the simple diffusion flux, J_{UWL}, of the substrate S from the bulk water phase of the contents of the intestinal lumen up to the enterocyte surface across the UWL, and 2) the transfer of S through the epithelium, J_{epith}, that has two components in parallel (5, 19, 45, 53): 2.1) a non saturable passive mechanism, perhaps through a paracellular way, J^{p}_{epith}, and 2.2) a non-passive saturable carrier mediated transport, phloridzin sensitive, J^{np}_{epith}.

In the steady-state (53):

$$J_{UWL} = J_{epith} = J^{p}_{epith} + J^{np}_{epith}$$

Under this condition a concentration of the substrate at the aqueous-membrane interface, S_m, lower than that in the bulk phase, S_o, will be established. The difference $S_o - S_m$ will depend on the UWL resistance to S diffusion and will become zero in the absence of UWL. Assuming a steady-state:

$$J_{UWL} = J_{epith} = V$$

$$J^{p}_{epith} = V_D \quad \text{and}$$

$$J^{np}_{epith} = V_T$$

This means that:

$$V = J_{UWL} = J_{epith} = J^{p}_{epith} + J^{np}_{epith} = V_D + V_T$$

The flux across the UWL is given by the expression:

$$V = \frac{D'}{\delta}(S_o - S_m) \qquad [1]$$

where δ is the UWL effective thickness (cm), and $D' = D\rho$, D, being the free diffusion coefficient of sugar at 38° C (cm^2·seg^{-1}) and ρ a conversion factor required for adaptation to the dimensions of S (mM) and V (nmoles·cm^{-1}·min^{-1}).

The passive flux across the epithelium, considering the subepithelial concentration of S as negligible, will be:

$$J^{p}_{epith} = V_D = K_D \cdot S_m \qquad [2]$$

where K_D is a true mass-transfer coefficient related to the permeability coefficient of the epithelium for the substrate S, with the dimensions derived from those of S_m and V_D.

At this stage the permeation rate by the non passive component, $J^{np}_{epith} = V_T$, can be described by the MICHAELIS-MENTEN equation,

$$V_T = \frac{S_m \cdot V_{max}}{S_m + K_m} \qquad [3]$$

in which, V_{max} and K_m are the «true» values of these kinetic parameters.

Therefore as in the steady-state $V = V_D + V_T$, the corresponding substitution for their values will yield:

$$V = \frac{D'}{\delta}(S_o - S_m) = K_D \cdot S_m + \frac{S_m \cdot V_{max}}{S_m + K_m} \quad [4]$$

The perfusion rate, PR ($ml \cdot min^{-1}$), modifies δ and thereby the UWL resistance and the S_m value. As PR increases, δ and $S_o - S_m$ will decrease up to a PR limit in which $\delta = 0$, $S_o = S_m$ and

$$V_{lim} = P \cdot S_o + \frac{S_o \cdot V_{max}}{S_o + K_m}$$

V_{lim} being the maximum substrate absorption rate theoretically attainable by increasing PR for each S_o value.

Obviously, in all situations in wich $\delta \neq 0$ the values of S_m, and not those of S_o, are to be taken to calculate K_D and K_m from [1], [2], [3] and [4]. If instead of S_m, the S_o are taken, biased apparent K'_D and K'_m values will be obtained, being $K'_D < K_D$ and $K'_m > K_m$. The differences in K_m values reported in the literature for the same sugar from experiments *in vitro* and those much higher between *in vitro* may be explained for differences in δ and S_m according to experimental techniques. In fact δ will reasonably be higher *in vivo* than *in vitro*, as in the last condition effective stirring diminishes the UWL thickness. The differences in K'_m shown in table II can be ascribed to the decrease in δ as the PR increases.

As the transport saturation rate corresponds to $1/S = 0$, V_{max} has to be the same independently of S_o or S_m values be taken. The figures 1 and 2 show this constancy of V_{max}.

On the other hand, $V_D = K_D S_m = K'_D S_o$ and

$$V_T = \frac{S_m \cdot V_{max}}{S_m + K_m} = \frac{S_o \cdot V_{max}}{S_o + K'_m}$$

Therefore

$$\frac{K_D}{K'_D} = \frac{S_o}{S_m} = \frac{K'_m}{K_m} \quad [5]$$

This expression shows that K'_D and K'_m result biased in respect to the true values of K_D and K_m according to S_o/S_m ratio, although in reciprocal sense.

Values for S_m may be obtained if δ or true K_m are known. However, some calculating methods (12, 26, 47, 49) can only be applied cautiously to *in vivo* experiments in intestine. Therefore it was deemed best to adopt as true K_m values for sugars the lowest ones found in the literature from *in vitro* experiments. Thus $K_m = 0.41$ mM (17) may be taken as true K_m for D-glucose in hamster jejunum and 0.83 mM (27) in rat jejunum. For D-galactose there is less information but enough to adopt 0.6 mM and 1 mM for hamster and rat respectively.

Consequently the following expressions can yield S_m, K_D and δ from V, V_D, V_T and K_m,

$$S_m = \frac{K_m \cdot V_T}{V_{max} - V_T} \quad [6]$$

$$K_D = \frac{V_D}{S_m} \quad [7]$$

and

$$\delta = \frac{D'(S_o - S_m)}{V} \quad [8]$$

Application of the model to the experimental results. If the assumptions for the model agree with reality, the following requirements must be fulfilled:

1. *K_D must not change at different perfusion rates*

In fact, K_D has to be dependent on the substrate and the epithelium charactheristics and not on S_m. Thus, it must be the same for all S_o and PR values. Calculation of K_D from the experimental results yields (mean ± s.d.)

		K_D
Rat	D-glucose	360.4 ± 45
	D-galactose	326.7 ± 31
Hamster	D-glucose	933 ± 112
	D-galactose	859 ± 43.2

The s.d. is in all cases low enough for the fulfilling of the K_D constant requirement to be accepted. Differences between the K_D for glucose and galactose are not significant.

2. *Linearity of the plot $1/V_T$ versus $1/V_D$*

As

$$\frac{1}{V_T} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \cdot \frac{1}{S_m},$$

and

$$S_m = \frac{V_D}{K_D},$$

then

$$\frac{1}{V_T} = \frac{1}{V_{max}} + \frac{K_m \cdot K_D}{V_{max}} \cdot \frac{1}{V_D},$$

K_m, K_D and V_{max} being independent of both S_o and PR. Figure 3 shows, for D-glucose absorption in rats, enough agreement between the experimental results and the theoretical predictions of the model. Similar agreement was observed with the other results in rat and hamster. The slope $K_m \cdot K_D/V_{max}$, amounts to 0.898, 0.972 (rats), 0.956 and 0.902 hamster) for glucose and galactose respectively, all of which practically coincide.

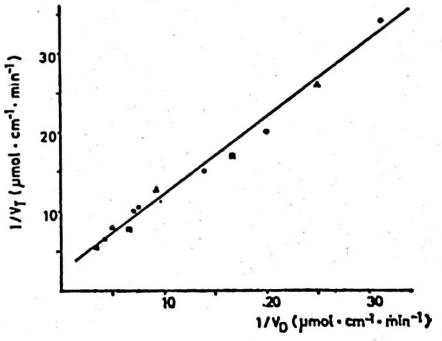

Fig. 3. *Plot of $1/V_T$ versus $1/V_D$ for glucose transport in the rat.*
● PR = 2.8, △ PR = 5.6, ○ PR = 13.5 and □ PR = 18.5 ml·min^{-1}.

3. *For each sugar and animal species, the quotient S_o/S_m is only dependent on PR*

According to the assumptions of the model [5], as K_m and K_D are constant for each sugar and animal, and K'_m and K'_D dependent on PR, S_o/S_m value will diminish when PR increases, but it must be kept the same throughout the different S_o values.

In table III this requirement is seen to be sufficiently met, and as in all cases the standard deviation of the mean is insignificant.

4. *The effective thickness of unstirred water layers, has to be dependent on the animal and on the PR, but not on the nature and concentration of the sugar.*

As UWL on the intestinal epithelium are neither flat nor cylindric, δ is not really the UWL thickness, but an operative quantity or «effective» thickness (8, 53), with the value ascribed in [1].

The free diffusion coefficient of D-glucose in water at the body temperature, D, has been estimated in $D = 8.49 \times 10^{-6}$ cm^2·seg^{-1} (21). The dimensions of S_o and S_m are mmoles·l^{-1} = 10^{-6} moles·cm^{-3}, and those of V are nmoles·cm^{-1}·min^{-1} = $= 10^{-9}/60$ a_{UWL} moles·seg^{-1}·cm^{-2}, being a_{UWL} the area (cm^2) of the UWL corresponding to 1 cm of jejunum length. Then, $\rho = D'/D = 60 \times 10^3 \times a_{UWL}$.

If the jejunum is assumed to be a cylinder, and its capacity is 0.063 cm^3·cm^{-1} in rat (33), a_{UWL} will approximately be equal to $2\pi\sqrt{\frac{0.063}{\pi}} = 0.9$ cm^2.

Therefore,

$$D' = \rho D = 60 \times 10^3 \times 0.9 \times 8.49 \times 10^{-6} = 458.4 \times 10^{-3},$$

and [8] yields for the effective thickness

$$\delta \text{ rat} = 4{,}584 \frac{S_o - S_m}{V} \ (\mu m)$$

Table III. *Estimated values of S_o/S_m ratios and of unstirred water layer thickness (δ) at different perfusion rates (mean ± S.D., δ in μm).*

Perfusion rate:		2.8	5.6	13.5	18.5 (ml · min⁻¹)
Rats					
Glucose	S_o/S_m	26.5 ± 2	19.4 ± 0.5	14.2 ± 0.6	10.13 ± 0.6
	δ	163.9 ± 17	120.6 ± 9	95.5 ± 9	75.3 ± 7
Galactose	S_o/S_m	20.5 ± 0.6	16.2 ± 0.6	12 ± 0.4	—
	δ	155.6 ± 11	111 ± 11	90.3 ± 8	—
Hamsters					
Glucose	S_o/S_m	59.5 ± 2.5	—	39.6 ± 3	—
	δ	180.5 ± 16	—	111.9 ± 10	—
Galactose	S_o/S_m	58.2 ± 0.4	—	37.2 ± 1.3	—
	δ	178.6 ± 12.7	—	113 ± 6.3	—

An analogous estimate for hamsters yields $a_{UWL} = 1.0$ cm², $D' = 509.4 \times 10^{-3}$ and

$$\delta \text{ hamster} = 5{,}094 \frac{S_o - S_m}{V} (\mu m)$$

As it can be seen in table III, the estimations of δ for the different perfusion rates in rat and hamster also fulfil satisfactorily these predicted requirements. The effective thickness becomes lower as the perfusion rate increases.

Discussion

In the last years several models and equations have been proposed to express non-passive transport across intestinal wall in the presence of unstirred water layers, with or without passive components (17, 20, 23, 43-45, 53, 54). The models have stated theoretically and experimentally the strong influences that changes in area and thickness of UWL and area of absorbing surface, the topographical distribution of carriers in the villi geometry and concomitance of a passive component, can produce on the absorption kinetics and on the estimated values for active and passive transport parameters.

However, application of the models to the experimental situation poses great difficulties for the correct estimate of the absorbing area (18, 23, 49, 54, 55), and the UWL thickness (12, 26, 47, 49), especially in the case of the small intestine (49, 54) and still greater ones concerning *in vivo* experiments. The evaluation of «true» K_m under such conditions becomes merely orientative.

As the present paper aimed to study the effect of the perfusion rate on the absorption kinetics, the opposite procedure was adopted: the most probable values for «true» K_m were taken from the available literature as starting point, in order to estimate the effective concentration of sugar at the aqueous-membrane interphase, S_m, for the different sugar concentrations, in the bulk phase, S_o, at different perfusion rates. Once the S_m was known it was possible to calculate approximately the true mass-transfer coefficient for the transepithelial passive component, K_D, and to obtain an orientative value for the UWL effective thickness, δ.

The model adopted, based on assumptions for the steady-state (53), are readily acceptable for the purpose of this work. Absorption periods of 1 minute seem to be sufficient to attain the steady-state since absorption rates in a series of 1 minute consecutive periods, in equal conditions

are practically constant for more than 30 minutes under continuous single pass perfusion (33). On the other hand, in the rank of sugar concentrations used, the differences $V - V_D$, between total absorption and the remaining one in the presence of phloridzin can be taken as a measure of the active transport component, V_T. In fact the double reciprocal plot of $1/V_T$ vs $1/S_o$ shows an acceptable linearity. At the perfusion rates chosen, significant changes either in the absorbing surface area (54) or in blood flux (23) are not to be expected.

The experimental results meet satisfactorily the requirements of the model, giving it validity under the adopted experimental conditions.

LINEWEAVER-BURK lineal transformation of MICHAELIS-MENTEN equation yields the same V_{max} value at the different perfusion rates, as it had been reported (16, 17, 35, 49). These V_{max} values are similar to those previously obtaided for the same animal species and sugars in the laboratory (22).

The estimates of S_m show the relevance of unstirred water layers in intestinal absorption kinetics, as they offer a high diffusion resistance yielding very high S_o/S_m ratios. As perfusion rate increases, this resistance and the S_o/S_m ratio decrease, with enhancement of the absorption rate by simultaneously increasing the passive and the non-passive fluxes. Depending on the perfusion rate, S_o/S_m can reach up to 26.5 in rat and 59.5 in hamster.

The consideration of UWL resistance as a rate-limiting pass for intestinal absorption (13, 35, 44, 50) has been confirmed, since for the same sugar concentration in the bulk phase, absorption increases as UWL resistance decreases.

As expected, values of δ become lower as the perfusion rate increases. Previous estimates of UWL thickness *in vivo* (13, 17, 35, 37, 47, 49, 51) yielded δ higher values than those here reported, e.g. WINNE (54) obtained values of 330-380 μm using a perfusion rate of just 0.5 $ml \cdot min^{-1}$, which is less than 1/5 the lowest one (2.8 $ml \cdot min^{-1}$) used in the experiments here reported. Although the relation between PR and $1/\delta$ is not lineal, extrapolation to 0.5 $ml \cdot min^{-1}$ would yield values not far from the aforesaid.

As regards K'_D, an apparent mass-transfer coefficient for passive diffusion (V_D/S_o), it increases with the perfusion rate as it had been reported (7, 45, 49, 56). Instead, K_D values estimated as V_D/S_m, seem to be acceptably independent of PR. Differences between K_D for D-glucose and D-galactose as well in rat as in hamster are non-significant.

It is striking, however, that K_D estimates for rat were much lower than those for hamster suggesting a higher passive permeability in the last species. As V_D values are similar for both animal species (table I), the difference in K_D must be due to S_m values, much higher in rat than in hamster. Besides real differences in passive permeability and morphological peculiarities with diverse epithelial surface area per centimeter of intestine length, a possible explanation might be due to the adopted values for the K_m in rat (0.83 and 1.25 mM), which perhaps were higher than the real ones. If the K_m in both species were more alike, differences in both S_m and K_D would be found to be smaller.

In any case, but especially when working *in vivo*, apparent kinetic constant values are of little significance if the results are not corrected for the passive flux through the epithelium, and the experimental conditions affecting unstirred water layer resistance, particularly the perfusion rate, are not well specified.

Acknowledgements

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Resumen

Se estudia el efecto de la velocidad de perfusión sobre la cinética de la absorción de glucosa y galactosa de yeyuno de rata y hamster *in vivo*. La absorción total (V) y la absorción en presencia de florricina 0,5 mM (V_D) fue medida en períodos consecutivos de 1 minuto, y su diferencia, $V-V_D$, ha sido tomada como medida del transporte activo. Los valores de velocidad de flujo estudiados han sido 2,8, 5,6, 13,5 y 18,5 $ml \cdot min^{-1}$, y las concentraciones de azúcar en la solución de perfusión fueron 2, 5 y 10 mM.

Los resultados para las diferentes velocidades de flujo muestran un valor común de V_{max} para el mismo azúcar y especie animal. Se obtienen distintos valores para la constante de transporte, K'_m, y se calculan los coeficientes de difusión K'_D. Cuando la velocidad de flujo aumenta, K'_m decrece mientras K'_D incrementa.

Se asume que en el estado de equilibrio la difusión a través de las capas de agua no agitadas (UWL) es igual a la suma de una difusión pasiva y un transporte activo transepitelial. Este modelo está de acuerdo con los resultados. Se han calculado la concentración de azúcar en contacto con la membrana del enterocito (S_m), el espesor efectivo de UWL (δ) y el coeficiente de difusión real (K_D). La relación S_o/S_m y δ disminuyen cuando la velocidad de flujo aumenta.

Dependiendo de la concentración de azúcar, el componente pasivo es igual o más alto que el transporte mediado, tanto en rata como en hamster. La difusión a través de las capas de agua no agitadas resulta ser velocidad limitante en la absorción intestinal de azúcares.

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