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Kinetics of Intestinal Sugar Transport, *in vivo*

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Sugar absorption by the small intestine has been studied in rat and hamster *in vivo*, with luminal perfusion, during 1 minute successive periods. Transport is calculated as the difference between absorption and diffusion. The diffusion component is evaluated in the presence of phlorizin or as absorption of sorbose. The resulting K_T values for glucose and galactose (rat: 7.7 and 10 mM; hamster: 10 and 14 mM) and 3-0-methyl-glucose (hamster: 25-33 mM) are quite lower than those previously obtained *in vivo*, but still higher than those *in vitro*.

The physiological levels of glucose in the intestine of normally fed animals imply that the diffusion component plays an important role in the proximal regions of the small intestine, especially in rat.

It is well known that when the intestinal transport of non-electrolytes is studied *in vivo*, the results differ considerably from those obtained *in vitro*, mainly in relation to the apparent transport constant, K_T (5, 6, 16, 28, 32), and to their dependence on the Na^+ concentration in the bathing solutions (14).

As sugar absorption *in vivo* includes active transport and diffusion processes (9-11, 15), kinetics studies must take this fact into account prior to suggesting differences between transport systems working under *in vitro* or *in vivo* experimental conditions.

The present study was undertaken to investigate the kinetics of active sugar absorption in rat and hamster *in vivo*, under conditions apt to distinguish the diffusion and active transport components.

In addition, the glucose levels in gastrointestinal luminal content from normally fed animals have been estimated to assess the physiological significance of diffusion and transport in absorption.

Materials and Methods

Absorption. The experimental technique used has already been described elsewhere (26). Briefly, albino rats (Wistar strain) of 150-230 g and Golden hamsters of 75-100 g, of either sex, were anesthetized with intravenous injection of 12 % urethane (1 ml/100 g) after a 24 hour fast. Laparotomy was performed and a section of the jejunum was isolated be-

tween two glass cannulae connected to a perfusion system equipped with a peristaltic pump. After rinsing with 0.9 % NaCl, the intestine was perfused with Krebs-Henseleit-Bicarbonate (19), pH = 7.4 containing the sugars under study, i.e. glucose, galactose, 3-0-methyl-glucose or sorbose.

Experiments were carried out either by recycling the solution with a perfusion rate of 5.6 ml/min or without recycling at 2.8 ml/min. A number of successive absorption periods of 1-5 minutes were carried out in each animal.

Sugar absorption was estimated as the difference between the sugar present in the solution before and after the perfusion, and expressed in nmol/cm intestine/min. Aldohexose concentrations were measured following NELSON-SOMOGYI (24, 33), and sorbose following ROE (18, 29).

The estimation of operational kinetic parameters of absorption processes was done graphically after LINEWEAVER-BURK (22).

Glucose levels in gastro-intestinal content of normally fed rats and hamsters. The animals were killed at different times during the day and the stomach and small intestine were rapidly removed. The small intestine was divided into four segments of the same length in rat and into two in hamster. The contents of the different parts were emptied into centrifuge tubes. After centrifugation at 3000 rpm at 4° C for 15 min, samples were taken from the supernatant and enzymatically tested for glucose (31) in a Spectrophotometer Aba-100 (Abbot).

Results

Kinetics of the intestinal absorption of glucose, galactose and sorbose in rat in vivo. The intestinal absorption of glucose and galactose plotted against its concentration in the perfusion solution did not yield saturation curves (fig. 1-3). Although all these curves showed inflexion at low luminal sugar concentrations, they became straight lines at higher concentrations.

In agreement with previous observations (9-11, 15) the shapes of these curves suggest the existence of at least two compo-

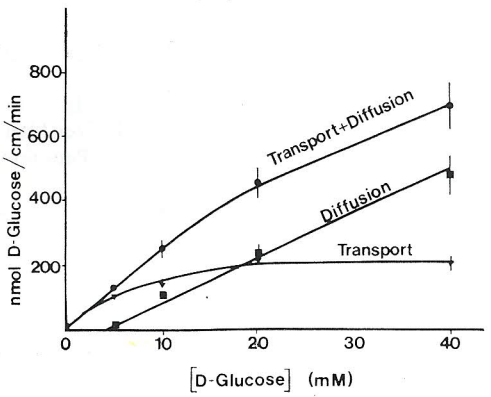

Fig. 1. *Kinetics of intestinal glucose absorption* in vivo *in rat.*
The experiments were carried out without recycling the solution, at perfusion rate of 2.8 ml/min and successive absorption periods of 1 min. Each point represents the mean of six determinations. Vertical bars are standard errors. Diffusion curve obtained in the presence of 5.10^{-4} M Phlorizin. Transport curve calculated from the difference between absorption (transport + diffusion) and diffusion.

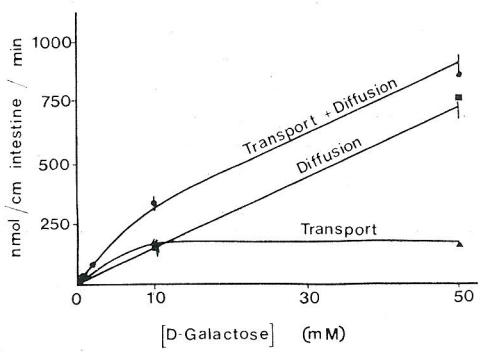

Fig. 2. *Kinetics of intestinal galactose absorption in rat* in vivo.
Experiments and symbols as in fig. 1.

nents in the absorption process, a saturable process upon which is superimposed a diffusion component.

The diffusion component of the absorption process of the sugars actively transported can be assessed by comparing in the same intestinal segment the absorption of an actively absorbed sugar, either *a*) with the absorption of the same sugar under conditions blocking the active component, or *b*) with the absorption of a sugar not actively transported having the same apparent diffusion constant (K_D). Phlorizin is a well known competitive inhibitor of sugar active transport (1) with very easily reversible effects (25). When the active component was blocked by phlorizin (5×10^{-4} M) the resulting absorption of glucose and galactose showed a linear relationship with their concentration in the perfusion solution whether the experiments were done by recycling the perfusion solution or not (fig. 1-2). The difference in the amount of sugar absorbed in the presence and absence of phlorizin at each luminal concentration reflects the amount of sugar passing the intestinal wall via an active transport mechanism.

Figure 1 shows that in the presence of phlorizin the absorption of glucose at a luminal concentration equal to or below 5 mM was not different from zero. Under these conditions glucose had to move against a concentration gradient, since its concentration in blood was approximately 5 mM in rat.

The diffusion component for glucose was also evaluated using the second method mentioned above. Since glucose and sorbose have similar diffusion constants (3, 11, 17) L-Sorbose was used as a non-transported sugar. The results obtained are shown in figure 3. The absorption of sorbose is proportional to its luminal concentration, as expected from a sugar absorbed by diffusion. The results obtained for glucose were not qualitatively different from those shown in figure 1.

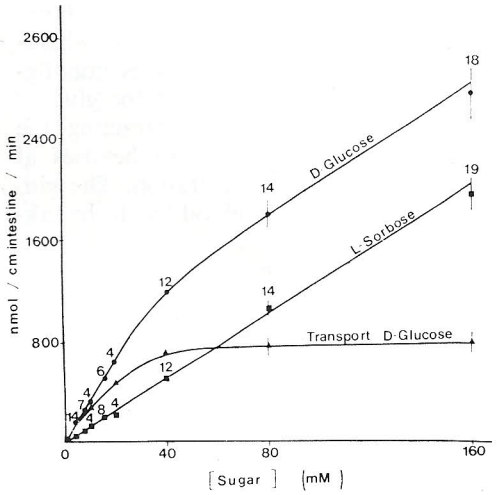

Fig. 3. *Kinetics of intestinal glucose and sorbose absorption in rat* in vivo.
The experiments were done by recycling the solution at a perfusion rate of 5.6 ml/min. Absorption periods were of 5 min. The figures on the points represent the number of determinations for each point and the vertical bars the standard error. Transport curve calculated from the difference between the glucose absorbed et each luminal concentration and the sorbose absorbed when its luminal concentration was 5 mM lower than that of glucose (see text).

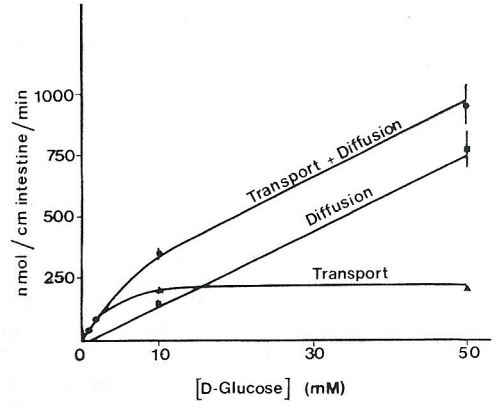

Fig. 4. *Kinetics of intestinal absorption of glucose in hamster* in vivo.
Experiments and symbols as in fig. 1. Seven determinations for each point.

Since the absorption of sorbose takes place at any luminal concentration whereas the diffusion of glucose does not (figure 1), the active component for glucose cannot be estimated by subtracting the observed absorption of both hexoses at the same luminal concentration. The glucose concentration in blood has to be taken into account and its active component is given by the difference between the amount of glucose absorbed at each luminal concentration and the absorption of sorbose observed when its luminal concentration was 5 mM lower than that of glucose.

The transport data, calculated after

Table I. *Apparent K_T and V_{max} values for the transport of glucose, galactose and 3-0-methyl-glucose in rat and hamster* in vivo, *after correction for the diffusion component of absorption.*

	Corrected for:	L-Sorbose		5×10^{-4} M Phlorizin	
		K_T (mM)	V_{max} (nmol/cm/min)	K_T (mM)	V_{max} (nmol/cm/min)
Perfusion with recycling					
D-Glucose	rat	17	800	16	240
D-Galactose	rat	—	—	20	240
Single pass perfusion					
D-Glucose	rat	—	—	7.7	250
	hamster	9	650	10.0	400
D-Galactose	rat	—	—	10.0	200
	hamster	20	1000	14.0	350
3-0-M-Glucose	hamster	25	350	33.0	500

Table II. *Glucose levels found in the gastrointestinal luminal content in rat and hamster, under normal conditions of feeding and sacrificed at different hours.*
Mean values accompanied by their standard errors. The figures in brackets represent the number of animals used in the study. «I» represents an intestinal segment.

		Glucose (mM)				
			Intestine			
Animal	Timetable	Stomach	I_1	I_2	I_3	I_4
Rat (5)	9	17.17 ± 1.58	88.66 ± 4.02	89.0 ± 2.75	15.03 ± 0.92	4.7 ± 0.37
Hamster (4)		12.00 ± 1.63	13.86 ± 3.31		8.8 ± 2.7	
Rat (5)	12	48.62 ± 7.70	83.10 ± 7.72	79.10 ± 3.76	9.98 ± 1.67	4.5 ± 0.20
Hamster (4)		18.50 ± 5.10	14.61 ± 3.00		6.40 ± 0.64	
Rat (5)	16	24.12 ± 4.90	68.00 ± 10.20	42.40 ± 2.07	16.62 ± 0.94	5.2 ± 0.31
Hamster (4)		4.10 ± 1.43	3.16 ± 1.10		2.10 ± 0.50	
Rat (5)	19	24.50 ± 0.90	42.30 ± 1.76	56.50 ± 0.90	15.00 ± 2.48	5.50 ± 0.47
Hamster (4)		14.00 ± 0.60	9.00 ± 0.50		6.50 ± 0.63	

correction for the diffusion component of absorption by both methods, when plotted against the concentration of sugar in the perfusion solution showed saturation kinetics (fig. 1-3) and allowed the estimation of the kinetic parameters for the active mechanism listed in table I.

Kinetics of the intestinal absorption of glucose, galactose, 3-0-methyl-glucose and sorbose in hamster, in vivo. Since smaller apparent transport constant, K_T, were obtained in rat when perfusion was carried out without recycling (table I), the kinetics of sugar absorption in hamster was undertaken under this condition.

As was observed in rat, the absorption of glucose, galactose and 3-0-methyl-glucose increased gradually upon increasing luminal concentration, but without saturation of the absorption process (fig. 4-7), suggesting again the coexistence of the two aforesaid components, transport and diffusion.

The active component for each sugar was evaluated following the double meth-

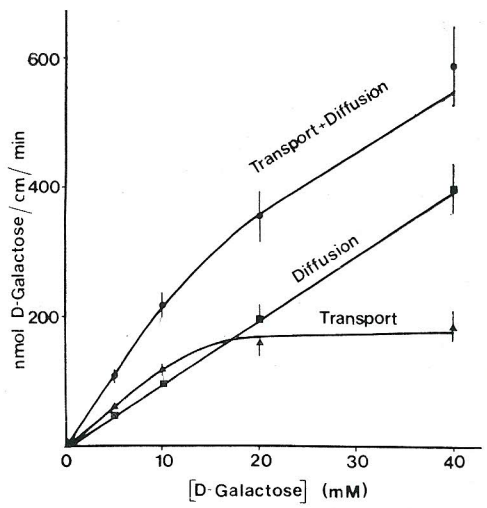

Fig. 5. *Kinetics of intestinal absorption of galactose* in vivo *in hamster.*
Experiments and symbols as in fig. 1. Six determinations for each point.

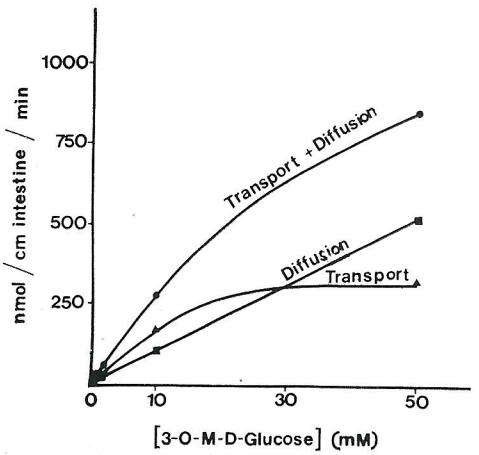

Fig. 6. *Kinetics of intestinal absorption of 3-0-methyl-glucose in hamster* in vivo.
Experiments and symbols as in fig. 1. Four determinations for each point.

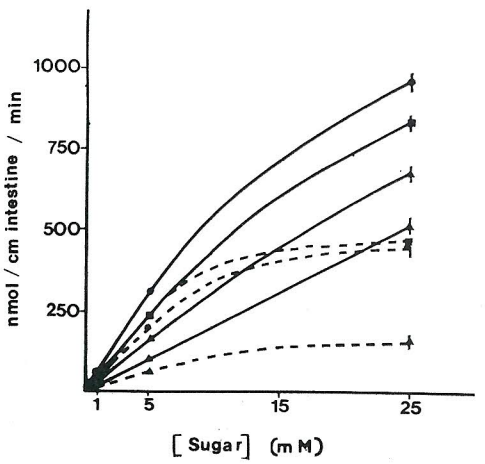

Fig. 7. *Intestinal absorption of glucose (■), galactose (●), 3-0-methyl-glucose (▲) and sorbose (△) in hamster* in vivo.
The experiments were carried out without recycling the solution, at perfusion rate of 2.8 ml/min and successive absorption periods of 1 min. Each point represents the mean of four determinations. Vertical bars are standard errors. Transport curves (---) calculated from the difference between sugar absorption (——) and diffusion (absorption of sorbose).

od described for rat. When the diffusion component for glucose was estimated by the absorption of sorbose, blood glucose level, about 6 mM in hamster, was taken up as in rat.

The apparent transport constant, K_T, and V_{max} obtained for each sugar, after correction for the diffusion component are listed in table I.

Glucose levels in intestinal content. Rats and hamsters normally fed with a usual mixed diet, were taken from the laboratory colony at 9, 12, 16 and 19 hs, and killed to know the glucose concentration in the stomach and in the luminal content of several regions of the small intestine. As table II shows, in rat glucose levels are moderate in stomach, high in the first moiety of the small intestine and low in the second one. In hamster, glucose levels, however, are rather low.

Discussion

Previous kinetics studies on the intestinal absorption of sugars *in vivo* in rat (6, 9-11, 15, 21, 23, 27) and dog (2) had shown the existence of both diffusional and saturation processes.

The results presented here for rat confirm those previous observations. In hamster the absorption of glucose, galactose and 3-0-methyl-glucose occurred by the two distinct pathways also observed in rat. One exhibits saturation kinetics, is phlorizin-sensitive and presumably represents the active hexose transport mechanism, whilst the second pathway is non-saturable and phlorizin-insensitive, features indicating passive diffusion.

When the absorption data were corrected for the diffusion component and plotted against the luminal sugar concentration, they showed typical saturation kinetics and allowed the estimation of the kinetics parameters (K_T and V_{max}) of the active mechanism.

The transport system affinity for glucose and galactose appeared to be similar for both rat and hamster (see table I). The affinity for 3-0-methyl-glucose was not estimated in rat, but in hamster it was found to be lower than for the other studied sugars.

The estimated Michaelis constant (K_T) for glucose in rat and hamster was found to be independent of the method used to evaluate the diffusion component, provided the perfusion rate and other experimental conditions remained constant. The absorption of glucose in the presence of phlorizin showed a good correlation with the absorption of sorbose. This indicates that both sugars have similar diffusion constants, K_D, in both rat and hamster. However the apparent transport constant for galactose and 3-0-methyl-glucose showed slight differences depending on whether their diffusion component was estimated by comparison with absorption of sorbose or by phlorizin blocking the active component (table I). These differences can be explained by the fact that the apparent diffusion constants for these sugars were in the series galactose $>$ glucose $=$ sorbose $>$ 3-0-methyl-glucose in both the animal species studied.

According to these results, the nature of the experimental conditions is an important factor in the determination of kinetic parameters. The estimates of the apparent transport constant, K_T, were lower when derived from perfusion experiments without recycling, probably due to the use of much shorter absorption periods and to lower changes in luminal sugar concentration along the intestinal segment (26) under this condition.

Although the K_T values for glucose, galactose and 3-0-methyl-glucose reported in the present study are lower than those previously found in *in vivo* experiments for glucose and galactose in rat, they are still higher than the values from *in vitro* experiments in rat and hamster (6-11, 15, 20, 30). Perhaps the *in vivo/in vitro* differences may be partially explained by

differences in the effective thickness of the unstirred layers, which is an important factor, as pointed out by several authors (12, 13, 28, 34-39). The thickness of these layers is surely lower in *in vitro* experiments because the bathing solutions are always well stirred. In *in vivo* experiments, instead, owing to the arrangement of the mucosa with its folds, villi and microvilli pointing towards lumen, the effective thickness of the unstirred layers must be much greater, even if the intestine is perfused. The presence of these layers, as it is well known (13, 34-39), reduces the apparent coefficient of permeability, while it increases the apparent transport constant in proportion to the thickness.

The results shown here support the idea that the transport systems of sugars *in vitro* possess the same properties as those *in vivo*. The *in vitro/in vivo* differences are chiefly explained by the incidence of physiological factors in the physical conditions in which these systems have to work, mainly those related to the thickness of the unstirred layers, to the energy availability of the cells, and to the withdrawal of the absorbed sugar by the blood circulation.

The real physiological meaning of the sugar transport process in a normal animal poses an important question. Our kinetic data show that diffusion by itself accounts for more than 50 % of the absorbed sugar when hexose concentration in lumen largely exceeds the blood value. The data in table II show that glucose concentration —which is the main sugar derived from food in the digestive tract— in the proximal half of the small intestine in rat is, as previously reported (34), sufficiently high as to make the diffusive pathway quantitatively more important for absorption than the active transport pathway. In hamster, however, these concentrations are quite low, and therefore passive sugar movement is less important than in rat.

Although the relative contributions of diffusion and active transport to the absorption of monosaccharides derived from food in normal animals, cannot be estimated from the present data, there is no doubt that diffusion has a significant role, especially in the proximal regions of the small intestine. The significance of the intestinal sugar transport systems, which may vary a lot according to animal species, food diet, intestinal motility, and other factors, consists in providing an additional passage for sugar, which might be less important than diffusion in the proximal small intestine, but acquires a growing importance as the sugar concentration in lumen decreases, and renders the absorption of sugars complete before it reaches the ileocecal valve.

Resumen

Se estudia *in vivo* la absorción de azúcares en intestino delgado de rata y hamster, con perfusión luminal, durante períodos sucesivos de 1 min. Se calcula el transporte como diferencia entre absorción y difusión. El componente de difusión se valora en presencia de florricina o como absorción de sorbosa. Se obtienen K_T para glucosa y galactosa (rata: 7,7 y 10 mM; hamster: 10 y 14 mM) y 3-0-metilglucosa (hamster, 25-33 mM) bastante inferiores a los previamente referidos *in vivo*, aunque todavía más altos que *in vitro*.

Los niveles fisiológicos de glucosa en intestino de animales alimentados con dietas ordinarias implican que el componente de difusión tiene considerable importancia en la primera parte del intestino, especialmente en rata.

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