REVISTA ESPAÑOLA DE FISIOLOGIA, 46 (4), 343-352, 1990

Use of the Paracellular Way for the Intestinal Absorption of Sugars

J.-J. Gato-Peciña* and F. Ponz**

Departamento de Fisiología Animal
Universidad de Navarra
31008 Pamplona (Spain)

(Received on July 20, 1990)

J.-J. GATO-PECIÑA and F. PONZ. *Use of the Paracellular Way for the Intestinal Absorption of Sugars.* Rev. esp. Fisiol., 46 (4), 343-352, 1990.

Passive absorption of D-Galactose (in the presence of 0.5 mM phlorizin), 2-deoxy-D-glucose and D-Mannitol by rat jejunum has been measured *in vivo* by perfusion of an intestinal segment with recirculation, along successive absorption periods of 5 or 10 min duration. In the range of 1 to 40 mM concentrations, the three solutes were absorbed at a very similar rate that varied as a lineal function of the concentrations in the perfusion solution. Absorption of 1 mM solute was not modified by the presence of 40 mM glucose or galactose. Passive absorption kinetics suggests processes of simple diffusion or solvent drag. The use of paracellular way for the passive absorption is supported by the fact that triaminopyrimidine (TAP) and protamine, which decrease the permeability through the tight junctions, also inhibit the absorption, with similar characteristics for both actions: TAP inhibition (53 %) is very rapid and can be easily reversed, while that of protamine (30 %) requires some time of previous exposure, lasts longer and can be reversed by heparin. The same analogy is shown by two actions that enhance the paracellular permeability: theophylline increases (30 %) the passive absorption with lasting effect, while luminal hypertony enhances absorption transitorily. The passive absorption of the assayed solutes could be estimated to take place by the paracellular way in at least 50 % and probably 70 % or even more. The measure of net fluid fluxes reveals that solute fluxes must be prevailingly explained by simple diffusion, as the solvent drag can only play a very minor role.

Key words: Intestinal absorption, Paracellular pathways, Sugars, Epithelial permeability.

As soon as the small intestine epithelium was classified as leaky (8, 14), the paracellular way through the *zonulae occludens* and intercellular spaces provided a partial explanation for the hydrophilic nutrient intestinal absorption (17). In fact, transepithelial net fluxes of isotonic solutions (59), as well as of some electrolytes and non electrolytes which are extracellular «markers», such as lanthanum (38, 67), mannitol, lactose, inulin or polyethylene glycol (42, 65) have been shown *in*

* Actual address: Departamento de Alergología, Clínica Universitaria. 31008 Pamplona (Spain)
** To whom all correspondence should be addressed.

vivo and *in vitro*. The potential contribution of the intercellular tight junctions to sugar intestinal absorption has been admitted or encouraged (2, 35, 49, 57, 58). *In vivo* absorption of glucose, galactose and other transportable sugars is known to comprise saturable Na-sugar cotransport processes at the enterocyte apical membrane, and simultaneously other non saturable ones, which are a lineal function of the luminal solute concentration and may use the paracellular way (11, 25, 58).

The use of the paracellular way for the non-mediated passive absorption of sugars is strengthened here, under a different approach. First, the lineal kinetics for the passive absorption of 2-deoxy-glucose, D-galactose and D-mannitol by the rat small intestine *in vivo* is established. Then, the effects of several tight junction modifiers are tested, with the result that triaminopyrimidine (17, 27, 41, 46, 61) and protamine (5, 16, 52, 54, 60) which decrease paracellular permeability, also diminish the passive absorption, while theophylline (4, 12, 21, 34, 46) and luminal hypertony (1, 10, 13, 33, 37, 62) increasing permeability also increment absorption. More than 50 % of the non saturable component of the sugar passive absorption involves passage through the paracellular way, by simple diffusion or solvent drag.

Materials and Methods

Wistar rats weighing 145-250 g, 24 hours fasted, were anesthetized with urethan, 0.125 mg/100 g. An about 20 cm long segment of proximal jejunum was perfused *in vivo* after the PONZ *et al.*, technique (53) by means of a peristaltic pump (Harvard Apparatus). The perfusion fluid was recirculated through the jejunal segment at a rate of 5.6 ml/min during successive absorption periods of 5 or 10 min duration. The perfusion solutions were Krebs-Ringer-Tris (63, phosphate replaced by Tris) or 0.9 % NaCl, adjusted at pH 7.4 excepting in TAP experiments. The initial volume of the perfusion fluid in the reservoir was always 10 ml, accurately measured. At the end of each absorption period, air was pumped through the perfusion system to collect all the residual fluid, which was rapidly weighed (± 5 mg) to determine the final volume. Between an absorption period and the next one, the intestinal lumen was rinsed by pumping first saline solution and then air.

The absorption was measured as the difference between the amount of solute initially present in 10 ml of the perfusion solution and that recovered in the volume of intestinal perfusate at the end of each experimental period. Absorption rates are expressed in micromoles of solute per cm length of intestine (53) per 5 or 10 min. Solute concentrations were determined by radioactivity counted with a Beckman LS 1800 scintillation counter.

As passively absorbed solutes, 2-deoxyglucose, mannitol and galactose were chosen. With galactose, the active Na-sugar cotransport was blocked by 0.5 mM phlorizin (11, 58).

D-Glucose, D-Galactose, D-Mannitol (Merck), 2-Deoxy-D-Glucose, Theophylline, Protamin sulfate, Phlorizin (Sigma), Heparin (Rovi) and 2,4,6-Triaminopyrimidine (Sigma or Fluka) were used. The labelled compounds D-[1-^{14}C]-galactose, D-Deoxy-D-[1-^{14}C]-glucose were from DuPont, and D-Mannitol-[1-^{14}C] from Amersham.

Results

Kinetics of the passive absorption. — The passive absorption rate of the three compounds, 2-deoxyglucose, mannitol and galactose in the presence of phlorizin, was examined in the range of 1-40 mmol/l concentrations in recirculation perfusion periods lasting 5 minutes each. Up to 8 successive absorption periods were per-

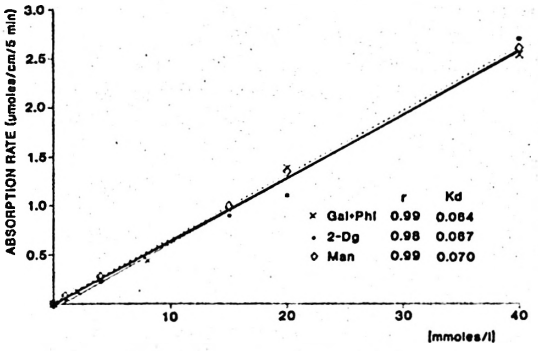

Fig. 1. *Passive absorption of galactose (+ 0.5 mM phlorizin), 2-deoxy-glucose and mannitol by rat jejunum as a function of luminal concentration. In vivo* perfusion, with recirculation. Absorption periods of 5 min. r, correlation coefficients of regression lines. K_D (slope), mass transference coefficients. Points, mean from 5 to 27 experimental data.

formed in the same jejunal segment, with equal or different concentrations. With the same concentration, the absorption rate along the 8 periods was kept practically constant, with a mean error lower than ± 7.5 %.

As figure 1 shows, the absorption rate for each of the three solutes appears to vary as a lineal function of the luminal concentration. The regression lines adjusted well to the experimental points with correlation coefficients higher than 0.98. The absorption values for the three compounds were very similar, as could be expected from their similar molecular size. The mass transference coefficients, K_D, were practically coincident.

Such a good linearity suggested the presence of fluxes due to simple diffusion or solvent drag, although they could also be explained by the use of a membrane transporter of very low affinity. Taking into account our experimental error, the Michaelis-Menten kinetics in the range of 1-40 mM would not be distinguishable from a straight line if the apparent Km values were 500 mM or higher.

To test if a transporter was involved, absorption experiments with 1 or 4 mM 2-deoxy-glucose in the presence of 40 mM galactose, and with 1 or 4 mM galactose in the presence of 40 mM glucose were carried out. In all cases (table I) the sugar absorption rate at low concentration was not modified by the presence of other sugars at 10 or 40 times higher concentrations. Consequently, the use of a common transport system for the observed passive absorption remains very unlikely.

Passive absorption inhibition by triaminopyrimidine (TAP). — The effects of 15 mM TAP added to the perfusion fluid at pH 6.1 on the passive absorption of 20

Table I. *Passive absorption of galactose and 2-deoxyglucose at low luminal concentrations, in the presence of another sugar at high concentration*

Means ± SE. Number of data, in parenthesis. n.s. = non significant. (Gal, Galactose; 2-Dg, 2-deoxyglucose; Glc, Glucose; Phl, phlorizin).

Sugar	Other sugar	Passive absorption (µmoles/cm/5 min)	
Gal + Phl	1 mM -	0.064 ± 0.003	(8)
	1 mM + 40 mM Glc	0.061 ± 0.001	(9) n.s.
	4 mM -	0.185 ± 0.011	(9)
	4 mM + 40 mM Glc	0.206 ± 0.012	(9) n.s.
2-Dg	1 mM -	0.055 ± 0.003	(12)
	1 mM + 40 mM Gal	0.051 ± 0.002	(12) n.s.
	4 mM -	0.220 ± 0.015	(9)
	4 mM + 40 mM Gal	0.200 ± 0.004	(11) n.s.

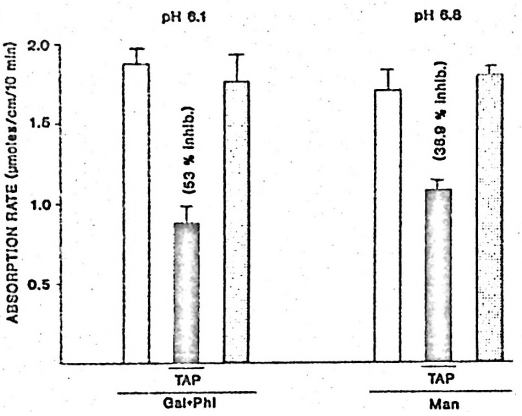

Fig. 2. *Effects of 15 mM 2,4,6-triaminopyrimidine (TAP) on passive absorption of 20 mM galactose (+ 0.5 mM phlorizin) and 20 mM mannitol across rat jejunum,* in vivo.
Means ± SE, n = 14 (Gal) and 6 (Man).

mM galactose in 10 min periods are shown in figure 2. TAP inhibited 53 % sugar absorption. Since the more active monoprotonate form of TAP becomes predominant at pH 6.1, this value was adopted (40, 41).

Other experiments with 20 mM mannitol, at pH 6.8, with a low proportion of the monoprotonate form of TAP, revealed a 36.9 % inhibition (fig. 2).

In both cases, the TAP inhibitory effect did not increase from previous exposure of the intestine to 15 mM TAP for 10 minutes and was easily and completely reversed by washing the intestinal lumen with saline solution.

Inhibition by protamine. — The effects of protamine on the passive absorption of D-galactose and 2-deoxy-D-glucose are shown in figure 3. When added to the sugar solution at 100 μg/ml concentration, protamine did not affect absorption. However, if the jejunal segment was perfused for 15 minutes with the same protamine concentration before the absorption period, an about 30 % inhibition of the 2-deoxyglucose and D-galactose passive absorption was observed.

At 1000 μg/ml concentration, protamine produced strong desquamation and other epithelial damages.

In some experiments, the effects of protamine (100 μg/ml) on the active plus passive absorptions of 20 mM galactose without phlorizin addition, were assayed. A lower inhibition value (10-16 %) was

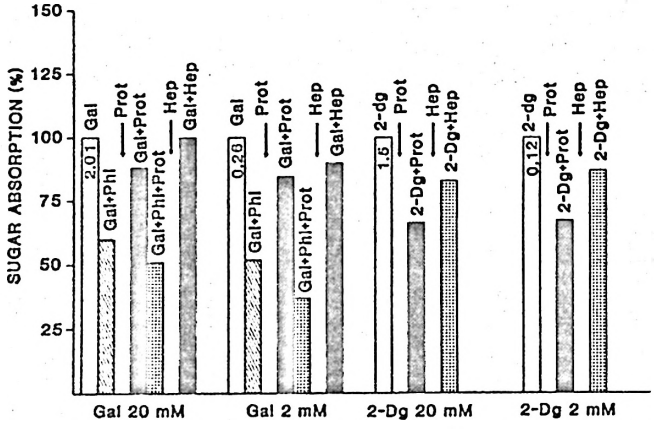

Fig. 3. *Effects of protamine (100 μg/ml) on galactose and 2-deoxy-glucose absorption across rat jejunum,* in vivo.
Partial reversion by heparin (30 μg/ml). Arrows signify exposures to protamine (15 min) or heparin (10 min). For each series, absorption in the first condition is given in μmoles/cm/15 min and taken as 100 for the other conditions. n = 6-13 (2-Dg), n = 8-16 (Gal).

found, in correlation with the fact that under those conditions the passive component amounts to 50 % or more of the total absorption.

The protamine inhibitory action could still be observed after washing the jejunal lumen with perfusion solution. But if the washing solution contained heparin (15-30 µg/ml), the inhibition of the passive sugar absorption was reduced by a 50-90 %. This partial reversion may be explained by the ability of heparin to form a complex with protamine, and be related to the reversion of the protamine induced changes in the structure of the tight junctions (5).

Passive absorption increase by theophylline. — Experiments were made with 20 mM galactose (in the presence of 0.5 mM phlorizin), adding 5 mM theophylline to the sugar solution (fig. 4). The passive absorption of galactose increased 28 % above the control. This effect was also observed in a sequent absorption period after thoroughly washing the intestinal lumen with saline solution.

As theophylline inhibits the sugar transport across the enterocyte basolateral membrane (24, 30, 44), the action of luminal 5 mM theophylline on the absorption of 1 mM galactose (in the absence of phlorizin) was tested. Under these conditions, the transcellular active transport component is very prevalent (25), but significant differences by theophylline were not found.

Increase of passive absorption by luminal hypertony. — The effect of hyperosmolarity on the passive absorption of 20 mM galactose and 20 mM 2-deoxy-glucose was assayed. The control perfusion fluid containing 20 mM sugar was practically isotonic (0.32 osm/l), and D-mannitol was added up to 0.52 osm/l to obtain a hypertonic solution. When the perfusion fluid with the sugar was hypertonic, the passive absorption rose about 50 % above that with isotonic solution. A previous exposure of the jejunal segment to the hypertonic solution for 10 minutes, did not modify the subsequent sugar pas-

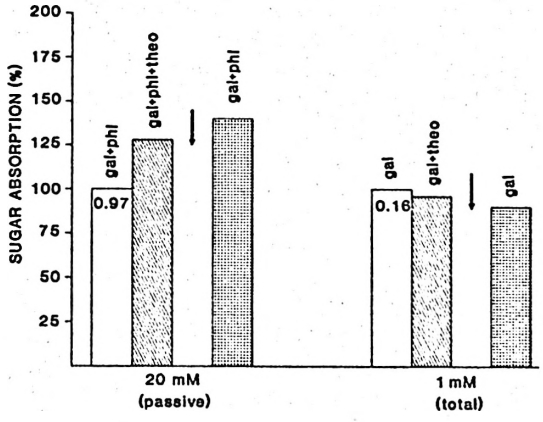

Fig. 4. *Effects of 5 mM theophylline on 20 mM galactose passive absorption, and on 1 mM galactose total absorption.*
Arrows signify thorough washing with saline solution. For each series, absorption in the first condition is given (µmoles/cm/10 min) and taken as 100. n = 4-9.

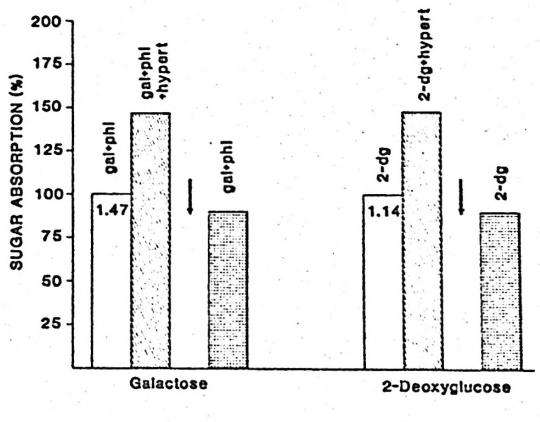

Fig. 5. *Effects of luminal hypertony (0.52 osm/l) on passive absorption of 20 mM galactose and 20 mM-2-deoxyglucose.*
Arrows signify washing with saline solution. For each series, absorption in the first condition is given (µmoles/cm/10 min) and taken as 100. n = 5-9 (2-Dg), 9-3 (Gal).

sive absorption in isotonic medium. On the other hand, if after the increase in passive absorption obtained by luminal hypertony, the jejunum was washed with isotonic saline solution, the next absorption period under control conditions gave normal values of sugar absorption.

Discussion

Kinetics. — Measuring the passive absorption of D-galactose (in the presence of 0.5 mM phlorizin), 2-deoxy-D-glucose and D-mannitol by the solute disappearance from the perfusate may be considered admissible as the three compounds are scarcely or not at all metabolized by the epithelium. Moreover, opposite fluxes to the lumen in so short experimental periods can be disregarded as those solutes are not normally present in blood, and they are rapidly extracted from the villi by the circulatory system to be distributed into all the extracellular fluid of the body. After eight consecutive 5 minute long absorption periods, the total quantity of a 20 mM solute absorbed by a 20 cm long jejunal segment may be estimated in about 150-160 μmoles (8 × 0.96 μmoles × 20 cm), and would produce, without considering renal excretion, assuming a 30 % body weight as extracellular fluid volume, a solute concentration lower than 4 mmoles/l.

The kinetics of passive absorption rate as a function of luminal solute concentration (1-40 mM) fits well with non significantly different straight lines, with slope K_D values which are coincident in the range of the experimental errors. This linearity suggests mechanisms of simple diffusion, solvent drag, or mediated transport with very low affinity. As mentioned before, the last possibility may be disregarded as the Km would be too high ($\geq$ 500 mM), and as the passive 1 mM sugar absorption was not inhibited by the presence of another sugar at 40 mM concentration. Moreover, mannitol is generally considered as an inert non transportable solute that can be used as extracellular marker.

Solvent drag through the paracellular way has been recently vindicated as a prominent mechanism for intestinal passive absorption of sugars and other non-electrolytes (2, 35, 49), according to *in vivo* long lasting experiments (hours) with perfusion of 50-80 cm of small intestine, in the presence of actively transportable 20-30 mM nutrients. About 0.2 reflexion coefficients were estimated for sugars (47, 50). In our experimental conditions, however, without activation of the fluid flux by transportable nutrients, the fluid net flux from the mucosal to the serosal side, measured as the difference between the initial and the final volume for each period of 5 minutes, was always lower than 0.3 ml for a jejunum segment about 20 cm long. Net flux of solute, J_S, by solvent drag can be estimated as $J_S = J_V (1-\sigma) \times \bar{c}$, where J_V is the solvent flux, σ is the reflexion coefficient for the solute and c is the mean concentration of solute in the channel. With a solvent flux of 0.3 ml/5 min, even taking $\sigma = 0$, and $\bar{c} = 20$ mM, the solute flux due to solvent drag would be $J_S = 0.3$ ml × 20 μl/ml/5 min = 6 μl/5 min. If $\sigma = 0.2$, J_S would be correspondingly lower, 4.8 μl/5 min. Actually, the net flux of solute measured along 5 min of passive absorption by a 20 cm long jejunum segment was three or four times greater (18-20 μmoles). This allows to relegate the solvent drag to an absorption minor component, while diffusional fluxes appear to play the major role. This conclusion, as will be seen later, is strongly reinforced by the experiments with luminal hypertonic solutions.

2,4,6-Triaminopyrimidine (TAP). — TAP reduces paracellular permeability in several epithelia (17, 27, 41, 46, 61). At pH 6.1, 15 mM TAP inhibited 53 % the passive absorption of galactose and at 6.8 it inhibited 37 % the mannitol absorption.

Similar results have been reported in dog ileum (32) and rat jejunum (66). The effects of TAP on the passive absorption may be explained by its binding to ionic groups in the Na^+ channels of the tight junctions (40, 41). Both inhibitions are exerted immediately, and can be easily reversed by washing the intestine with saline solutions. The somewhat higher inhibition found at pH 6.1 may be due to the greater proportion of monoprotonate form of TAP at that pH than at pH 6.8 (40, 41).

As the paracellular Na^+ conductance is inhibited about 85 % by 10-15 mM TAP (40, 41) and the passive absorption of galactose is only inhibited about 50 %, a part of the solute flux must take place by TAP non-sensitive ways in the same tight junctions, or across the enterocyte membrane.

Protamine. — This basic protein, as other cationic polypeptides, increases tight junction resistance and reduces paracellular permeability (5, 16, 52, 54, 60). The inhibition of the passive absorption of galactose and 2-deoxy-glucose by protamine was about 30 %. A certain time of action on the mucosa was required before these effects could be manifested. Saline washing was unable to reverse the inhibition, but a partial reversion was obtained if the washing solution contained heparin. These characteristics are also found when protamine increases the transepithelial resistance and reduces the paracellular permeability (28, 36, 48). The delay in the apparition of the last mentioned effect has been attributed to an indirect action of protamine: changes in the tight junctions would follow other ones initiated on the cell membrane (16, 52, 56).

Theophylline. — From the luminal side, theophylline increases the paracellular pathway permeability to water, chloride (20, 46), salicylate, urea, antipyrine (4), 3-*o*-methylglucose (34) and saccharose (26), due to structural changes in the tight junction induced by the rise in intracellular AMPc (12).

Passive absorption of galactose increases about 30 % if 5 mM theophylline is added to the perfusion fluid containing the sugar. This effect persists in the following absorption period without theophylline. These results may be explained by the changes in the tight junctions induced by this substance, that increases the paracellular conductance to chloride (3, 18, 20) without affecting that of Na^+ (3). Accordingly, the paracellular transfer of galactose would use, besides the TAP sensitive Na^+ channels, other paths in the junctions utilized by Cl^- flux, which become enhanced by theophylline.

Contrariwise, if an important concurrent transcellular flux of galactose was present, its inhibition by theophylline had to be expected as it inhibits the sugar transporter at the enterocyte basolateral membrane (24, 29, 55).

Hypertony. — A moderate hyperosmolarity (0.52 osm/l) in the perfusion fluid gives place to marked increases (50 %) in the passive absorption of galactose and 2-deoxyglucose. The effect appears immediately and is completely reversed by washing the intestine and returning to isotonic solutions.

These results are consistent with those evidencing similar increases in transepithelial fluxes of several extracellular markers such as lanthane (13, 55), mannitol or innulina (33, 39, 51), saccharose (6, 15, 64), and peroxydase (10, 37) produced by mucosal hypertony, which are being ascribed to a diminution in number of links between the filaments of tight junction structures, expanding their clear spaces (37).

Final volume measures of the perfusate showed that, as it was expected, a net flux of fluid from serosal to mucosal, amounting to 0.3-0.4 ml/10 min/20 cm took place

during the 10 min periods of absorption with hyperosmotic solutions. In spite of this flux of volume, an important opposite flux of galactose and 2-deoxy-glucose from mucosal to serosal was simultaneously present, that cannot obviously be a consequence of solvent drag, but requires diffusional processes.

Importance of the paracellular way in sugar absorption. — The results here reported allow us to conclude that passive absorption of D-galactose, 2-deoxy-glucose and D-mannitol takes place to a high degree through the paracellular way. TAP and protamine, that diminish tight junction permeability, also decrease passive absorption, while theophylline and mucosal hypertony that increase paracellular permeability, also enhance passive absorption. Nevertheless, some transcellular passive flux cannot be excluded, because some hydrophilic substrates similar in size to those here tested are able to enter the brush border membrane vesicles (22, 23, 31, 45) or isolated enterocytes (30) by simple diffusion.

The proportion in which passive absorption takes place across the paracellular way is difficult to know as no agent has been reported to annul this way. TAP inhibits 85 % of the paracellular conductance to Na^+ without affecting that to Cl^-. As sugar passive absorption is inhibited about 50 % by TAP, as the 30 % increases by theophylline suggest a coincident use of a different Cl^- way, and as transmembrane simple diffusion of sugars has generally been considered a minor possibility, it is likely that sugar passive absorption through the tight junctions occurs in at least at 70-80 %.

The physiological significance of the paracellular way in the intestinal absorption of sugars proceeding from usual meals constitutes a final consideration. *In vivo* experiments with rat intestine have shown that at 20-40 mM sugar concentrations, the Na-sugar cotransport is almost saturated, the phlorizin insensitive passive absorption being equal or superior to the transport fluxes. At higher luminal concentrations, passive absorption increases while cotransport remains stable (11, 25). It must be taken into account that after ordinary meals, 40 to 110 mM and 20 to 300 mM glucose concentrations were found in proximal jejunum of rats (9, 25, 43) and human intestine (7, 19), respectively.

Furthermore, the presence of sugars, aminoacids or other cotransportable substrates in the luminal solution has been recently reported (2, 35, 49, 50) to exerts regulatory responses on the tight junction structure, leading to important increases in the paracellular fluxes of fluid and solutes, in such a way that paracellular absorption of hydrophilic nutrients is considered as a substantial component of their total physiological absorption, especially at high luminal concentrations. Our results, in the absence of activating solutes, strongly emphasize the physiological significance of the paracellular way in the sugar intestinal absorption. However, under our experimental conditions, the paracellular fluxes of solutes seem to depend much more on simple diffusion through the tight junctions, than on solvent drag.

Acknowledgements

This work was in part supported by a Grant of the «Comisión Interministerial de Ciencia y Tecnología» (PB 86-0407) (Spain). J. J. Gato-Peciña has a scholarship from the «Asociación de Amigos de la Universidad de Navarra» (Spain).

Resumen

Se mide la absorción pasiva de D-galactosa (en presencia de florricina 0,5 mM), 2 deoxi-D-glucosa y D-manitol por yeyuno de rata *in vivo*, mediante perfusión de un segmento intestinal con recirculación, a lo largo de períodos de absorción sucesivos de 5 ó 10 minutos. Entre concentraciones 1 a 40 mM, los tres solutos se absorben a velocidad muy similar, que es función lineal de su concentración en la solución de perfusión. La absorción de un soluto 1 mM

no se modifica por la presencia de glucosa o galactosa 40 mM. La cinética de absorción pasiva sugiere procesos de difusión simple o de arrastre por disolvente. El uso de la vía paracelular para la absorción pasiva está apoyada por el hecho de que la triaminopirimidina (TAP) y la protamina, que reducen la permeabilidad por las uniones intercelulares, inhiben también la absorción, con parecidas características en ambas acciones: la inhibición de la absorción por el TAP (53 %) es rápida, y se reversibiliza fácilmente, mientras que la producida por la protamina requiere cierto tiempo de exposición, dura más y se reversibiliza por heparina. Lo mismo sucede con dos acciones que favorecen la permeabilidad paracelular: la teofilina aumenta (30 %) la absorción pasiva con efecto duradero, y la hipertonía luminal la aumenta (50 %) en forma fácilmente reversible. Se estima que la absorción pasiva de los solutos ensayados tiene lugar por la vía paracelular en al menos un 50 y probablemente en un 70 o más por ciento. La medida del flujo neto de líquido revela que los flujos de soluto se han de explicar de forma predominante por simple difusión, y que el arrastre por disolvente sólo puede jugar un papel muy minoritario.

Palabras clave: Absorción intestinal, Vía paracelular, Azúcares, Permeabilidad del epitelio.

References

1. Armstrong, W. Mc., Byrd, B. J., Cohen, E. S., Cohen, S. J., Hamang, P. H. and Myers, C. J.: *Biochim. Biophys. Acta*, 401, 137-151, 1975.
2. Atisook, K., Carlson, S. and Madara, J. L.: *Am. J. Physiol.*, 258, C77-C85, 1990.
3. Bakker, R. and Groot, J. A.: *Am. J. Physiol.*, 246, G213-G217, 1984.
4. Barnett, G., Hui, S. and Benett, L. Z.: *Biochim. Biophys. Acta*, 507, 517-523, 1978.
5. Bentzel, C. H., Fromm, M., Palant, C. E. and Hegel, U.: *J. Membr. Biol.*, 95, 9-20, 1987.
6. Biber, T. V. L. and Curran, P. F.: *J. Gen. Physiol.*, 51, 606-620, 1968.
7. Borgstrom, B., Dahlqvist, A., Lundh, G. and Sjovall, J.: *J. Clin. Invest.*, 36, 1521, 1957.
8. Clarkson, T. W.: *J. Gen. Physiol.*, 50, 659-727, 1967.
9. Cole, A.: *Nature*, 191, 502-503, 1961.
10. Cooper, M., Teichberg, Ch. B. and Lifshitz, F.: *Lab. Invest.*, 38, 447-454, 1978.
11. Debnam, E. S. and Levin, R. J.: *J. Physiol.*, 246, 181-196, 1975.
12. Duffey, M. E., Hainan, B., Ho, S. and Bentzel, C. J.: *Nature*, 294, 451-453, 1981.
13. Erlij, O. and Martines-Palomo, A.: In «Membrane Transport in Biology». (Giebisan, G., Tosteson, D. C., Ussing, H. H., eds.). Springer-Verlag, Berlín, 1978, 3, pp. 1-26.
14. Fordtran, I. S.: *J. Clin. Invest.*, 47, 884, 1968.
15. Franz, T. J. and Van Bruggen, J. T.: *J. Gen. Physiol.*, 50, 933-949, 1967.
16. Fromm, M., Palant, C. E., Bentzel, C. J. and Hegel, U.: *J. Membr. Biol.*, 87, 141-150, 1985.
17. Frömter, E. and Diamond, J. H.: *Nature*, 235, 9-14, 1972.
18. Heinz, E.: In «Mechanics and energetics of Biological Transport» (Kleinzeller, A., Springer, G. F. and Witmann, E. M. G., eds.). Springer-Verlag. Nueva York, 1978, pp. 28-30.
19. Holdsworth, C. D. and Dawson, A. M.: *Clin. Sci.*, 27, 371-379, 1964.
20. Holman, G. D. and Naftalin, R. J.: *J. Physiol.*, 290, 351-366, 1979.
21. Holman, G. D. and Naftalin, R. J.: *J. Physiol.*, 290, 367-386, 1979.
22. Hopfer, U.: *Am. J. Physiol.*, 236, F1-F8, 1977.
23. Hopfer, U., Nelson, K., Perrotto, I. and Isselbacker, K. J.: *J. Biol. Chem.*, 248, 25-32, 1973.
24. Hopfer, U., Sigrist-Nelson, K. and Murer, H.: *Ann. N. Y. Acad. Sci.*, 264, 414-427, 1975.
25. Ilundain, A., Lluch, M. and Ponz, F.: *Rev. esp. Fisiol.*, 35, 359-366, 1979.
26. Jacobson, H. R.: *Am. J. Physiol.*, 236, F71-F79, 1979.
27. Jansen, J. W. C. M., Fleren-Jakobs, A. M. M., De Pont, J. J. H. M. and Bonting, S. L.: *Biochim. Biophys. Acta*, 598, 115-126, 1980.
28. Katchalsky, A.: *Biophys. J.*, 4, (Suppl.) 9, 1964.
29. Kimmich, G. A.: *Fed. Proc.*, 40, 2474-2479, 1981.
30. Kimmich, G. A. and Randles, J.: *Am. J. Physiol.*, 237, C56-C63, 1979.
31. Kinne, R. and Murer, H.: In «Intestinal ion transport» (Robinson, J. W. L., ed.). MTP Press, Lancaster, U. K., 1976, pp. 79-95.
32. Kreys, G. J., Seeling, L. L. and Fordtran, J. R.: *Gastroenterology*, 72, 685-691, 1977.
33. Laker, M. F. and Menzies, I. S.: *J. Physiol.*, 265, 881-894, 1977.
34. Lucke, H., Kinne, R. and Murer, H.: In «Mechanics of Intestinal Secretion». (Binder, H. J., ed.). Liss, New York, 1979, pp. 111-116.
35. Machen, T. E., Erlij, D. and Wooding, F. B. P.: *J. Cell. Biol.*, 54, 302-313, 1972.

36. Mamelak, M., Wissig, S. L., Bogoroch, R. and Edelman, I. S.: *J. Membr. Biol.*, 1, 144-176, 1969.
37. Marcial, M. A. and Madara, J. L.: *Lab. Invest.*, 56, 424-434, 1987.
38. Martínez-Palomo, A., Erlij, D. and Bracho, H.: *J. Cell. Biol.*, 50, 277-287, 1971.
39. Menzies, I. S.: *Biochem. J.*, 126, 19-20P, 1972.
40. Moreno, J. H.: *J. Gen. Physiol.*, 66, 97-105, 1975.
41. Moreno, J. H.: *J. Gen. Physiol.*, 66, 117-128, 1975.
42. Munck, B. G. and Rassmussen, S. N.: *J. Physiol.*, 271, 436-488, 1977.
43. Murakami, E., Saito, M. and Suda, M.: *Experientia*, 33, 1469-1470, 1977.
44. Murer, H.: *J. Cell. Biol.*, 89, 805-810, 1976.
45. Murer, H. and Kinne, R.: *J. Membr. Biol.*, 55, 81-95, 1980.
46. Naftalin, R. J. and Simmons, N. L.: *J. Physiol.*, 290, 331-350, 1979.
47. Naftalin, R. J. and Tripathi, S.: *J. Physiol.* (London), 370, 409-432, 1986.
48. Nevo, A., DeVries, A. and Katchalsky, A.: *Biochim. Biophys. Acta*, 17, 536, 1955.
49. Pappenheimer, J. R.: *J. Membr. Biol.*, 100, 137-148, 1987.
50. Pappenheimer, J. R. and Reiss, K. Z.: *J. Membr. Biol.*, 100, 123-136, 1987.
51. Pérez-González, M. and Whittembury, G.: *Pflügers Arch.*, 351, 1-12, 1974.
52. Poler, S. M. and Reuss, L.: *J. Gen. Physiol.*, 86, 42a, 1985.
53. Ponz, F., Ilundain, A. and Lluch, M.: *Rev. esp. Fisiol.*, 35, 97-104, 1979.
54. Quinton, P. M. and Philpott, C. W.: *J. Cell. Biol.*, 56, 787-796, 1973.
55. Rawlins, F. A., González, E., Pérez-González, M. and Whittembury, G.: *Pflügers Archiv.*, 353, 287-302, 1975.
56. Reuss, L. and Finn, A.: *J. Membr. Biol.*, 25, 191, 1975.
57. Robinson, J. W. L. and Antonioli, J. A.: *Gastroenterol. Clin. Biol.*, 4, 78-86, 1980.
58. Rodríguez, M. J., Ortiz, M., Vázquez, A., Lluch, M. and Ponz, F.: *Rev. esp. Fisiol.*, 38, 397-402, 1982.
59. Schultz, S. G.: *Yale J. Biol. Med.*, 50, 99-113, 1977.
60. Seiler, M. W., Rennke, H. G., Venkatachalam, M. A. and Cotran, R. S.: *Lab. Invest.*, 36, 48-64, 1977.
61. Specht, W.: In «Intestinal Permeation» (Kramer, M. and Lauterbach, F., eds.). Excerpta Medica, Amsterdam, 1977, pp. 4-40.
62. Turnhein, K.: In «Handbook of Experimental Pharmacology» Vol. 70/I: «Pharmacology of Intestinal Permeation» (Csáky, T. Z., ed.). Springer-Verlag, Berlin, 1984, pp. 381-451.
63. Umbreit, W. W., Burris, R. M. and Staffen, J. F.: In «Manometric Techniques». Burgess Publ., Minneapolis, 1959.
64. Ussing, H. H.: *Ann. N. Y. Acad. Sci.*, 137, 543-555, 1966.
65. Van Os, C. H., de Jong, M. D. and Slegers, J. F. G.: *J. Membr. Biol.*, 15, 363-382, 1974.
66. Vinardell, M. P. and Bolufer, J.: *Rev. esp. Fisiol.*, 39, 193-196, 1983.
67. Whittembury, G. and Rawlins, F. A.: *Pflügers Arch.*, 330, 302-309, 1971.