Hydrolysis-Dependent Absorption of Disaccharides in the Rat Small Intestine (Chronic Experiments and Mathematical Modeling)

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Abstract. In order to throw light on the mechanisms responsible for the enzyme-dependent absorption of disaccharides membrane hydrolysis of maltose and trehalose and the absorption of glucose (free and that derived from disaccharides) were studied in isolated loops (20 cm) of the rat small intestine in chronic experiments The rates of glucose absorption were 0 26–0 81 μmol min^{-1} cm^{-1} when the loop was perfused with a 12 5 to 75 0 mmol/l free glucose solution, which is only insignificantly higher than the rates observed during perfusion with equivalent maltose solutions The coupling coefficient (the ratio of glucose absorption rate to the rate of disaccharide hydrolysis) decreased from 0 90 to 0 60 with the increasing maltose concentrations in the infusate from 6 25 to 37 5 mmol/l, but remained unchanged ($\approx$0 95) within the same range of trehalose concentrations The permeability of the pre-epithelial barrier was equivalent to that of unstirred water layer of less than 40 μm thickness Fluid absorption was within the range of 0 73–2 55 μl min^{-1} cm^{-1}, and it showed a correlation with the rates of glucose absorption The results agree with a model developed on the assumption that free glucose and that released from disaccharides share the same membrane transporters It could be concluded that a close coupling of disaccharide hydrolysis with derived glucose absorption in chronic experiments is achieved mainly due to a high activity of glucose transporters, which are presumably not associated with membrane disaccharidases The transcellular active transport is a predominant mechanism of disaccharide-derived glucose absorption under conditions close to physiological

Key words: Small intestine — Membrane digestion — Glucose absorption — Mathematical modeling

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Introduction

Being one of the major food components in the diet of mammals, carbohydrates enter the body mainly in the form of poly- and oligosaccharides During luminal and membrane digestion in the small intestine they are split to monosaccharides, mostly to glucose, which then penetrates through the enterocytes into the internal medium of the body (Alpers 1987, Ugolev and Iesuitova 1991) Previous studies by Ugolev and co-workers (1985, 1986) have shown that under physiological conditions this is a highly efficient process due to close coupling between disaccharide membrane hydrolysis and absorption of released monomers It has been assumed that the coupling may be explained by the operation of a special enzyme-dependent system which provides a direct transfer of glucose released from disaccharides to the transporter of the enzyme-transport complex, without glucose dissipation in the bulk phase (Ugolev et al 1986, Riby et al 1993) According to *in vitro* experiments, on the other hand, free glucose and that derived from disaccharides form a common pool in the brush border region and seem to share the same transporters during their transfer across the apical membrane of the enterocytes (Metel'ski 1986, Gromova et al 1992) If this is the case, it remains unclear how dissipation of monomers in the bulk phase is prevented and how a close coupling between hydrolysis and absorption processes in chronic experiments is achieved, having in mind that the permeability of so called "unstirred layer" in the small intestine of unanesthetized rats is high (Anderson et al 1988, Strocchi and Levitt 1991)

It has been assumed recently that the high efficiency of the digestive-transport conveyor for poly- and oligosaccharides is provided due to paracellular transfer of glucose by solvent drag, which is considered as a major contributor to glucose absorption under normal conditions (Pappenheimer 1993) However, according to the above hypothesis, estimates of the maximal rate of active glucose transport have mainly been obtained from acute *in vivo* experiments (Pappenheimer 1990, 1993, Pappenheimer and Reiss 1987), whereas the results of chronic experiments in unanesthesized rats show much higher values of active glucose transport (Ugolev et al 1985, 1986, 1995, Gromova and Gruzdkov 1993)

This work examines the relationships between disaccharide hydrolysis and derived glucose absorption in the rat small intestine under conditions as close to physiological as possible To achieve this, the rates of hydrolysis of disaccharides (maltose and trehalose), the absorption of glucose (both free and that released from disaccharides), and transepithelial fluid fluxes were measured during *in vivo* perfusion of the isolated intestinal loop in chronic experiments The degree of coupling between hydrolysis and transport processes, and the permeability of the pre-epithelial layer were also evaluated The experimental data were compared with the mathematical model developed on the assumption that free glucose and that derived from disaccharides share the same membrane transporters during their transfer across the

apical membrane of the enterocytes Also, the model takes into consideration the influence of the pre-epithelial barrier and transepithelial fluid fluxes

Materials and Methods

Experimental technique

Surgical procedures and post-operative treatment Chronic experiments were performed in 8 Wistar rats (males, body mass 150 200 g) with isolated intestinal loops The surgical technique and experimental conditions were similar to those described earlier (Ugolev and Zaripov 1979, Ugolev et al 1986, 1995, Gromova and Gruzdkov 1993) The animals were taken for the experiments a week after a loop of the small intestine (20 cm in length at a distance of 15 cm from the duodenum) was isolated

The animals were given water on the day following the surgery, and easy digestible food on day 3 Thereafter, beginning 1 5 2 weeks after the operation, they received standard diet Throughout the experiment, the isolated loop was perfused daily either with test solutions (during the chronic experiments) or with 25 mmol/l glucose solution for one hour (on days where nothing else was done) to maintain the functional capability of the mucosa and to delay its atrophy The body mass of the animals was also measured daily

The conditions and technique of chronic experiments Chronic experiments continued for 7 8 weeks after the operation The lumen of the intestinal loop was perfused either with glucose (12 5, 25 0, 50 0 and 75 0 mmol/l, sequentially) or disaccharide (maltose, trehalose) solutions of equivalent (reffered to glucose) concentrations The experiments were performed at a constant osmolality of the infusates To provide this, substrate solutions were prepared in Ringer solution with a reduced NaCl concentration (100 mmol/l), so that with the highest of glucose concentrations used (75 mmol/l) the osmolality of the perfusion solution was slightly lower than that of the standard Ringer solution At lower substrate concentrations (12 5–50 0 mmol/l) mannitol was added in amounts needed to keep isoosmolality of the infusate

In some experiments the intestinal loop was also perfused with glucose (25 and 75 mmol/l) in the presence (or absence) of phloridzin (1 mmol/l)

A peristaltic pump provided perfusion rates within the range of 0 5 0 6 ml/min, with a deviation during the experiment of less than 2% The solutions entered the intestinal loop pre-heated to 38 °C in an ultrathermostat After a preliminary 15 min perfusion with the substrate at a particular concentration, three samples (at 5 min intervals) were sequentially taken for biochemical analysis The volume of each sample was also determined

In order to account for a possible influence of temporary changes in the functional state of the animal on hydrolysis and absorption processes, an additional perfusion was performed at the end of each experiment as a repetition of the first

one. Averages of the data obtained were used for subsequent calculations. For the same purpose all experiments with glucose, maltose and trehalose were repeated 2–4 times in each of the animals during 1.5 months.

Analytical methods and calculations. Glucose concentration was determined by the glucose oxidase method, and total glucose and disaccharide (maltose or trehalose) concentrations (in mmol/l glucose) were measured by the anthrone method as described earlier (Ugolev et al. 1986).

The rates of disaccharide hydrolysis (J_h in μmol/min) and absorption of glucose, both free and released from disaccharides (J_a^f and J_a^d respectively, in μmol/min), as well as the rate of fluid absorption (J_w, in ml/min) in the isolated intestinal loop were calculated using the following equations:

$$J_h = C_{in}^d \cdot V_{in}/5 - (C_{out}^{d+gl} - C_{out}^{gl}) \cdot V_{out}/5,$$

$$J_a^f = C_{in}^{gl} \cdot V_{in}/5 - C_{out}^{gl} \cdot V_{out}/5,$$

$$J_a^d = C_{in}^d \cdot V_{in}/5 - C_{out}^{d+gl} \cdot V_{out}/5,$$

$$J_w = (V_{in} - V_{out})/5,$$

where C_{in}^d and C_{in}^{gl} is the disaccharide and glucose concentration, respectively, in the infusate (mmol/l glucose); C_{out}^{gl} is the glucose concentration in the outflowing perfusate as evaluated by the glucose oxidase method (mmol/l); C_{out}^{d+gl} is the total concentration of sugars in the outflowing perfusate determined by the anthrone method (mmol/l glucose); V_{in} and V_{out} is the volume of the inflowing and outflowing perfusate, respectively, determined at 5-min intervals (ml).

The coupling coefficient of hydrolytic and transport processes was estimated as the ratio of the rate of glucose absorption in the isolated loop to that of hydrolysis of the corresponding disaccharide (Ugolev et al. 1985, 1986).

After the final experiments the animals were sacrificed; the mass and the length of the isolated loops (under 1 g load), as well as their serosal surface area, were then measured.

All data are given as mean ± S.E.M, calculated from the summed results of 2–4 experiments in 8 animals. The data obtained from the experiments were treated statistically using the paired Student's t-test.

Mathematical simulation

Background and main assumptions. The complete radial mixing model (Amidon 1980) was considered as the most appropriate one. The isolated intestinal loop was approximated as a tube L cm in length perfused at the rate of v_{in} (ml/min) with test substrate at the initial concentration S (disaccharide) or C (free glucose)(mmol/l). The structural and functional characteristics of the tube were assumed to be invariable along its length, with no radial gradient of the substrate concentration in the

lumen of the tube. The active surface of the tube was assumed to be separated from the lumen by a diffusion barrier (the unstirred layer). The following assumptions were also made:

1. Membrane hydrolysis of disaccharides is described by the enzyme kinetics equation (Dixon and Webb 1979):

$$J_h = V_m \cdot S_m/(K_m + S_m), \tag{1a}$$

where J_h is the rate of disaccharide hydrolysis; S_m is the disaccharide concentration at the digestive surface; V_m is the maximal rate of disaccharide hydrolysis; K_m is the Michaelis constant.

2. Glucose absorption (both free and that derived from disaccaharides) is mainly determined by the permeability of the apical membrane of the enterocytes, and may be described by the widely used equation (Thomson and Dietschy 1984; Westergaard et al. 1986; Meddings and Westergaard 1989):

$$J_a = J_{max} \cdot C_m/(K_t + C_m) + k_d \cdot C_m, \tag{1b}$$

where J_a is the rate of glucose absorption; C_m is the concentration of glucose (both free and that derived from disaccaharides) at the absorptive surface; J_{max} is the maximal rate of active glucose transport; K_t is the Michaelis constant for active glucose transport; k_d is the permeability of the brush border membrane for the passive glucose absorption.

3. Free glucose and that derived from disaccharide hydrolysis form a common glucose pool and share the same transporters during their transfer across the brush border membrane of the enterocytes (Sandle et al. 1983; Metel'ski 1986; Gromova et al. 1992).

4. Disaccharides and glucose move across the pre-epithelial layer both by diffusion and by solvent drag; the rate of this process, J_{pl}^{d} (for disaccharides) and J_{pl}^{gl} (for glucose), may be described by the modified Kedem-Katchalsky equation (Pappenheimer and Reiss 1987):

$$J_{pl}^{d} = k_1 \cdot (S - S_m) + k_2 \cdot (S + S_m) \cdot J_w \cdot 0.5 \tag{2a}$$

$$J_{pl}^{gl} = k_1 \cdot (C - C_m) + k_2 \cdot (C + C_m) \cdot J_w \cdot 0.5 \tag{2b}$$

where S and S_m, C and C_m are the concentrations of the corresponding substrates in the intestinal lumen and at the digestive-absorptive surface of the loop; J_w is the rate of fluid absorption; k_1 is the diffusion permeability of the pre-epithelial layer for the substrate; k_2 is the solvent drag coefficient; (to simplify the model it is accepted that the values of k_1, as well as those of k_2, are the same for disaccharides and for glucose).

5. The net transepithelial fluid flux J_w in the small intestine may be considered as the sum of three components:

$$J_w = J_{w0} + k_a \cdot J_{max} \cdot C_m/(K_t + C_m) - k_{os} \cdot (S + C - A) \quad (3)$$

where J_{w0} is the portion of the fluid flux independent of the presence of glucose in the perfusate; $k_a \cdot J_{max} \cdot C_m/(K_t + C_m)$ is the component of the fluid flux which depends on the rate of active glucose transport (according to the hypothesis proposed recently by Loo et al. (1996)); $k_{os} \cdot (S + C - A)$ is the component of the water flux which depends on the contribution of disaccharides and glucose to the total osmolality of the perfusate; S and C is the concentration of disaccharides and glucose, respectively, in the bulk phase; k_a and k_{os} are the corresponding constants; A is a constant introduced to take into account the fact that the experiments were performed at a constant osmolality of the initial infusates.

6. To take into account the inhibition of disaccharide hydrolysis in the presence of glucose (Sandle et al. 1983) (which has been assumed to be a competitive one) the following approximation of the value of K_m^* may be used:

$$K_m^* = K_m + C_m/Q \quad (4)$$

where K_m is the "true" Michaelis constant for disaccharide hydrolysis (without inhibition by glucose); C_m is the glucose concentration at the digestive-absorptive surface; Q is the ratio of disaccharide and glucose affinities for the enzyme; (for example, if $Q = 10$, the affinity of the enzyme for the disaccharide is 10 times higher than that for glucose).

Rough evaluation of the model parameters. To evaluate the permeability of the pre-epithelial barrier unstirred layer) an eqiuvalent resistance ($R_{eq}(j)$) of the epithelial and pre-epithelial layers was calculated for three glucose concentrations (12.5; 25.0; and 50.0 mmol/l) in the initial perfusate using the approach proposed earlier by other investigators (Levitt et al. 1987) with our modification (Gruzdkov and Gromova 1995):

$$R_{eq}(j) = \frac{L}{v_{in} \cdot \ln(C_{in}(j)/C_{out}(j))} \quad (j = 1, 2, 3) \quad (5)$$

where $C_{in}(j)$ and $C_{out}(j)$ is the glucose concentration (mmol/l) in the initial and the outflowing perfusate, respectively, corrected for the fluid absorption (secretion); L is the length of the isolated intestinal loop (cm); v^{in} is the rate of perfusion (ml/min).

The minimal value of eqiuvalent resistance (R_{eq}^{min}) was estimated by graphic extrapolation of $R_{eq}(j)$ to the region: $C_{in} \approx 0$. The R_{eq}^{min} was taken as a rough (overestimated) approximation for the resistance of the pre-epithelial layer (R_{pl})

and the $k_l = 1/R_{pl}$ as an approximation for its diffusion permeability (Eqs. 2a, 2b).

The value of $k_d \approx 1/R_{eq}$ was calculated from the data obtained during the perfusion of the isolated loop with glucose (75 mmol/l) in the presence of phloridzin (1 mmol/l), and was taken as an approximation of the passive permeability of the brush border membrane for glucose in Eq. 1b.

Approximated values of V_m and J_{max} (Eqs. 1a,1b) were also calculated from the experimental data using the following formulas:

$$V_m = J_h/L;\ J_{max} = J_a/L - k_d \cdot C, \tag{6}$$

where J_h and J_a are the rates of disaccharide (maltose or trehalose) hydrolysis and glucose absorption (μmol/min), respectively, in the isolated loop perfused with the maximal substrate concentration (75 mmol/l glucose); $C = (C_{in} + C_{out})/2$ is the average glucose concentration along the isolated loop (mmol/l); L is the length of the isolated loop (cm).

The values of these and the other parameters of the model (K_m^*, K_t, k_2, k_a, k_{os}, and Q) were then fitted by iteration to achieve the minimal values of the function: $\lambda = \sum(\delta x_j)^2$, $(j = 1, 2, 3, 4)$ where δx_j is the weighed difference between the model and experimental data for the rates of disaccharide hydrolysis or glucose absorption at four substrate concentrations in the infusate (C_j = 12.5; 25.0; 50.0 and 75.0 mmol/l glucose).

Results

Experimental findings

Body mass and morphometric measurements. The behavior of the rats and the amount of food consumption after the surgery suggested a normal physiological status of the animals during the whole experimental period (7–8 weeks). The body mass of the experimental animals, being 148±3 g (mean±S.E.M) before the operation, at first decreased to 139±4 g 6 days after the surgery, and steadily increased afterwards to reach 151±4 g and 211±8 g on day 11 and 52 respectively.

According to the measurements made 7–8 weeks after the operation the length of the isolated loop was 21.8±1.2 cm, the serosal surface area was 17.4±1.2 cm^2, and the mass of the loop was 1.23±0.09 g. Expressed per cm of intestinal length, the mass of the isolated loop was almost two times smaller than that of the intestine below the anastomosis (56.4±2.1 versus 99.8±4.2 mg/cm, $P < 0.01$). This may be accounted for by both atrophy (hypoplasia) of the isolated loop and hypertrophy of the remaining part of the intestine.

Hydrolysis of disaccharides and glucose absorption. The rates of disaccharide hydrolysis and glucose absorption in the isolated loop increased hyperbolically with

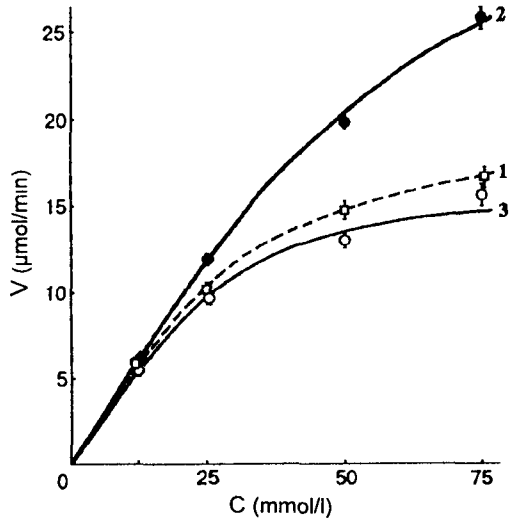

Figure 1. Hydrolysis of maltose (2) and absorption of free glucose (1) and that released from maltose (3) in the isolated loop of the rat small intestine in chronic experiments Abscissa substrate concentration (C) in the initial perfusate (mmol/l of glucose), Ordinate the rates (V) of maltose hydrolysis and glucose absorption (μmol/min) Points represent the experimental data (mean±S E M), the curves are the result of mathematical simulation

the increasing solute concentration in the infusates (Fig. 1). Even at the maximal concentration of disaccharides (37.5 mmol/l) or glucose (75 mmol/l) however, a complete saturation of the hydrolysis and absorption processes was not reached.

Similar to the experiments performed earlier with the same experimental technique (Ugolev et al. 1985, 1986, 1995), we observed a high absorption rate of glucose (both free and that released from maltose). At free glucose concentrations of 12.5 and 75.0 mmol/l the rates of its absorption were 20.2±0.57 and 58.4±3 3 μmol·h^{-1}·cm^{-2} (per cm^2 of serosal surface area of the loop), respectively; these values were 3 to 8 times higher than the maximal rate of active glucose transport observed earlier in *in vivo* experiments in anaesthesized rats (≈7.5 μmol·h^{-1}·cm^{-2} (Pappenheimer 1990)).

Within the whole range of solute concentrations used (12 5–75 mmol/l glucose) the absorption rate of glucose released from maltose hydrolysis was insignificantly lower ($P > 0.05$) than that of free glucose, substrate concentrations in the infusates being equivalent (Fig 1). At a low maltose concentration (6.25 mmol/l) most of the released glucose was absorbed and the coupling coefficient was rather high (0.904±0.010). However, in contrast to the previous data (Ugolev et al. 1985, 1986),

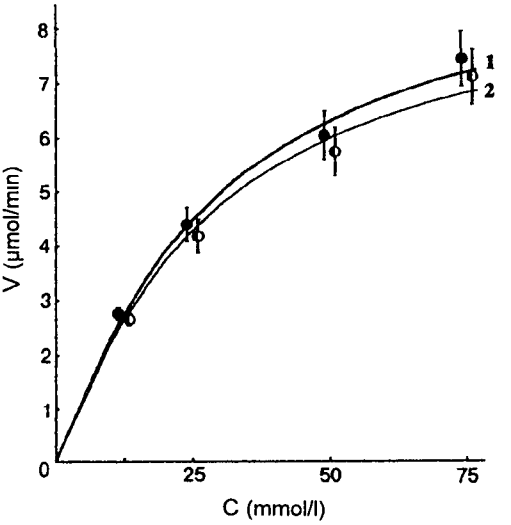

Figure 2. Hydrolysis of trehalose (1) and absorption of derived glucose (2) in the isolated loop of the rat small intestine in chronic experiments Abscissa trehalose concentration (C) in the initial perfusate (mmol/l) , Ordinate the rates (V) of trehalose hydrolysis and glucose absorption (μmol/min) Points represent the experimental data (mean±S E M), the curves are the result of mathematical simulation

we observed a steady decrease of the coupling coefficient with the increasing maltose concentration in the infusate (0 810±0 012, 0 653±0 016 and 0 600±0 011 at 12 5, 25 0 and 37 5 mmol/l, respectively)

The rates of trehalose hydrolysis and derived glucose absorption (Fig 2) were considerably lower than the corresponding values during maltose perfusion (Fig 1) Within the whole range of trehalose concentrations the coupling coefficient was rather high and practically invariable (0 950±0 004, 0 948±0 006, 0 945±0 005 and 0 951±0 006 at 6 25, 12 5, 25 0, and 37 5 mmol/l trehalose, respectively)

During the perfusion of the isolated intestinal loop with free glucose (25 and 75 mmol/l) in the presence of phloridzin (1 mmol/l) the sugar absorption was inhibited to 83 and 79%, respectively

Fluid absorption During the perfusion of the loop with isoosmotic solutions of the substrates the rate of fluid absorption increased with the increasing sugar concentration in the infusate (Fig 3) This was statistically significant ($P < 0\ 05$) in the case of free glucose perfusion when fluid absorption increased from 16 1±2 7 μl/min (at 12 5 mmol/l) to 46 9±7 0 μl/min (at 75 0 mmol/l) (Fig 3, curve 1)

A close correlation was observed between glucose (both free, and that derived from disaccharides) and water absorption The correlation coefficients were 0 994,

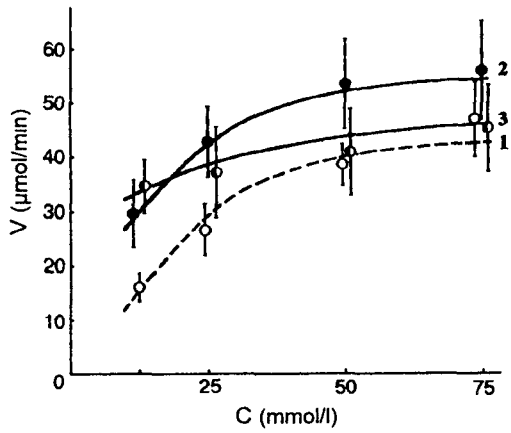

Figure 3. Fluid absorption in the isolated loop of the rat small intestine perfused with glucose (1), maltose (2), or trehalose (3) solutions in chronic experiments Abscissa concentration of substrates (C) in the initial perfusate (mmol/l), Ordinate the rate (V) of fluid absorption (μl/min) Points represent the experimental data (mean±S E M), the curves are the result of mathematical simulation

0.964; and 0.989 for free glucose, maltose and trehalose perfusions, respectively

Mathematical simulation

Permeability of the pre-epithelial layer. The values of $R_{eq}(J)$, calculated using formula (5) for three glucose concentrations in the infusate (12.5; 25.0 and 50 mmol/l), were 18.3, 28.0 and 54.0 min·cm^{-2}, respectively (per cm of intestinal length) Graphic extrapolation of $R_{eq}(J)$ to the region $C_{in} \approx 0$, gave a value of R_{eq}^{min} of about 12.5 min·cm^{-2} per cm of intestinal length (10.0 min·cm^{-1} per cm^2 of the serosal surface area). We took R_{eq}^{min} as a slightly overestimated value of the resistance of the pre-epithelial layer (R_{pl}). This is equivalent to the diffusion resistance of the unstirred water layer with the thickness of

$$d = R_{pl} \cdot D = 10.0 \cdot 6.7 \cdot 10^{-6} \cdot 60 \approx 40\ \mu m,$$

where D is the coefficient of glucose diffusion in water ($6.7 \cdot 10^{-6}$ cm$^2 \cdot$s^{-1}) (Westergaard et al. 1986; Levitt et al. 1987; Strocchi and Levitt 1991). This value of d is consistent with that obtained earlier by other investigators (Anderson et al. 1988; Strocchi and Levitt 1991) and by ourselves (Gruzdkov and Gromova 1995) in anaesthesized rats, but sharply differs from the previous estimations based on the results of acute experiments *in vivo* (for reviews see Thomson and Dietschy 1984; Hosoda 1992; Gruzdkov 1993).

Table 1. The main parameters of the model which describes disaccharide hydrolys is and glucose absorption in the isolated loop of the rat small intestine in chronic experiments

Substrate perfused	Simulated process					
	Disaccharide hydrolysis			Glucose absorption		Water absorption
	V_m μmol min^{-1} cm^{-1}	K_m mmol l^{-1}	Q dimen sionless	J_{max} μmol min^{-1} cm^{-1}	K_t mmol l^{-1}	J_{w0} μl min^{-1} cm^{-1}
Glucose			–	0 70	4 3	−0 15
Maltose	0 890	3 5	10	0 79	4 6	0 60
Trehalose	0 219	10 8	30	2 30	4 8	1 15

The kinetics of hydrolytic and transport processes The results of mathematical simulation of disaccharide hydrolysis and absorption of glucose and water in the intestinal loop are shown by the curves in Figs 1, 2 and 3 Table 1 shows those model parameters which were different in the simulation of glucose, maltose or trehalose perfusions of the intestinal loop The values of the remaining parameters of the model were $k_d = 0\ 002$ cm^2 min^{-1}, $k_1 = 0\ 1$ cm^2 min^{-1} $k_2 = 0\ 9$, $k_{os} = 1\ 0\ 10^{-5}$ cm^5 μmol^{-1} min^{-1}, $k_a = 3\ 0\ 10^{-3}$ cm^3 μmol^{-1} $v_{in} = 0\ 52$ ml min^{-1}, $L = 22$ cm

As can be seen from Figures 1–3, with these parameters the model yields results which are in a close agreement with the corresponding experimental data on the rates of disaccharide hydrolysis, and glucose and fluid absorption This closeness per se does not mean that the estimated values should be regarded as the "true" parameters of disaccharide hydrolysis and glucose absorption in the small intestine under our experimental conditions Rather, they are only probable ones However, a good agreement of the simulation with the experimental results does suggest that the model is adequate and may be applied for theoretical analysis

Discussion

A high efficiency of the hydrolysis-dependent glucose absorption in the small intes tine under normal conditions may be regarded as a well established fact (Ugolev et al 1985, 1986, Pappenheimer 1993) The coupling coefficient has been used for a quantitative evaluation of the digestive transport conveyor's efficiency (Ugolev et al 1985, 1986) It should be noted that the value of this parameter (calculated

as the ratio of glucose absorption rate to that of disaccharide hydrolysis) not only depends on molecular events in the vicinity of the brush border membrane, but also on the particular experimental conditions as well (the lenght of the isolated intestinal loop, the rate of perfusion, and the functional state of the animal during the experiment). However, under fixed conditions, the changes of the coupling coefficient upon varying disaccharide concentrations in the infusate may characterize the relationships between hydrolytic and transport processes. Thus, on the basis of the finding that at a low (13.8 mmol/l) and a high (55.0 mmol/l) maltose concentration in the infusate the coupling coefficient had almost the same value (0.90±0.09 and 0.87±0.07, respectively) the existence of a special enzyme-dependent system was suggested which provided for a direct transfer of glucose released from disaccharides to the transporter of the enzyme-transport complex, without glucose dissipation in the bulk phase (Ugolev et al. 1986).

The results of the present, more detailed, study show, however, that the coupling coefficient decreases steadily (from 0.904±0.010 to 0.600±0.011) with the increasing maltose concentration in the infusate from 6.25 to 37.5 mmol/l. This allows to suggest a saturation of the glucose transporters at a high disaccharide concentration. As a result, a considerable portion of maltose-derived glucose diffuses across the pre-epithelial barrier (unstirred layer) back into the intestinal lumen. Our data are in a good agreement with the hypothesis that free glucose and that derived from disaccharides form a common pool in the brush border region and seem to share the same transporters during their transfer across the apical membrane of the enterocytes (Sandle et al. 1983; Metel'ski 1986; Gromova et al. 1992). This is also supported by the fact of a slightly higher absorption of free glucose in the small intestinal loop in comparison with the absorption of glucose released from disaccharide hydrolysis (Fig. 1, curves 1 and 3). The model based on the above hypothesis allowed us to obtain results close to the corresponding experimental data, the value of J_{max} for active transport of glucose derived from maltose being only slightly different from that for active free glucose transport (0.79 and 0.70 $\mu mol \cdot min^{-1} \cdot cm^{-1}$, respectively) (Table 1). Thus, although specific glucose transporters are localized at the apical membrane of the enterocytes in a close vicinity of the enzyme maltase, they are not necessarily associated with the enzyme as the enzyme transport complexes or ensembles.

In the experiments with trehalose the coupling cofficient was effectively the same (about 0.95) within the whole range of disaccharide concentrations tested (from 6.25 to 37.50 mmol/l). On the one hand, this completely corresponds to Ugolev's hypothesis of enzyme transport complexes. Moreover, an agreement with experimental data was only achieved if the value of J_{max} for active transport of trehalose-derived glucose has been taken in the model more than 3 times higher than that for free glucose active transport (2.3 versus 0.7 $\mu mol \cdot min^{-1} \cdot cm^{-1}$). On the other hand, however, these facts cannot be regarded as direct or crucial evi-

dence for the existence of trehalase transporters complexes. Indeed, the results of the mathematical simulation show that, when disaccharidase activity is low and the capacity of the glucose active transport, on the contrary, is high (as in the case of trehalose perfusion in chronic experiments), one should expect a relatively low glucose concentration at the apical membrane of the enterocytes. The glucose transporters are far from being saturated and the exit of trehalose-derived glucose across the pre-epithelial barrier into the intestinal lumen is almost negligible. So, the model predicts that under these particular conditions, even without any direct interaction between trehalase and glucose transporters, the coupling coefficient must be rather high and almost independent of the trehalose concentration in the infusate.

Besides, there is an explanation for the different values of J_{max} predicted by the model for the absorption of free glucose, and the glucose released from disaccharides. As most other investigators, we considered the intestinal loop as a smooth cylindrical surface with an adjoining diffusion layer. However, it has been shown earlier (Gusev et al. 1983; Gruzdkov 1993) that peculiarities of the intestinal geometry (the existence of folds, villi, and microvilli) may significantly influence the apparent kinetic constants for membrane hydrolysis and transport of nutrients. It may be assumed that the above mentioned differences in the J_{max} values reflect (at least in part) variations in the distribution of glucose (free, and that released from disaccharides) along the intestinal villus and microvillus rather than possible co-operative interactions between the enzyme (trehalase) and glucose transporters.

The role of the pre-epithelial layer in the efficiency of hydrolysis-dependent glucose absorption in the small intestine has so far remained unclear. Its permeability estimated in this study is equivalent to the permeability of the unstirred water layer of less than 40 μm thickness. However, in spite of this rather high permeability, the coupling coefficient proved to be more than 0.90 at 6.25 mmol/l of maltose, and about 0.95 within 6.25–37.5 mmol/l of trehalose concentrations in the infusate. According to our data, such a degree of the coupling between disaccharide hydrolysis and glucose absorption may be provided mainly due to a high capacity of active glucose transport in chronic experiments. Indeed, the value of J_{max} =0.7 μmol$\cdot$min$^{-1}\cdot$cm^{-1} (52.6 μmol$\cdot$h$^{-1}\cdot$cm^{-2} per cm^2 of the serosal surface of the loop) for active glucose transport estimated by us in chronic experiments was about 7 times higher than that estimated earlier in acute experiments in rats (about 7.5 μmol$\cdot$h$^{-1}\cdot$cm^{-2})(Pappenheimer 1990, 1993). So, under conditions close to physiological within the range of normal concentrations of the sugars in the intestinal lumen (less than 30 mmol/l glucose (Ferraris et al. 1990; Diamond 1991)) the permeability of the apical membrane of the enterocytes for active glucose transport is of the same order as that of the pre-epithelial layer. The latter, at least in part, prevents the exit of the disaccharide-derived glucose from the brush border membrane into the intestinal lumen and, therefore, provides efficient operation of

the digestive-transport conveyor.

At first sight, a close correlation between glucose (both free and that derived from disaccharides)and water absorption which was observed by us in chronic experiments corresponds to Pappenheimer's hypothesis about the paracellular transfer of glucose by solvent drag as a major mechanism of its absorption under normal conditions (Pappenheimer and Reiss 1987; Pappenheimer 1990, 1993). However, at the highest maltose concentrations in the infusate (37.5 mmol/l) the maximal rate of fluid absorption under these conditions (J_w) is only 0 056±0 001 ml/min, whereas, according to the model estimations, the average concentration of maltose-derived glucose at the absorptive surface (C_{as}) of the loop does not exceed 20 mmol/l. Even on the assumption that glucose concentration in the intercellular space (C_{ic}) is three times higher (60 mmol/l) the solvent drag component of glucose absorption would be only:

$$J_{sd} = J_w \cdot (C_{as} + C_{ic}) \cdot 0.5 = 0.056 \cdot (20 + 60) \cdot 0.5 = 2.24 \ \mu\text{mol/min}.$$

This is less than 15% of the total glucose absorption in the loop (15.59±0.69 μmol/min). So, the close correlation between glucose and water absorption seems to indicate that the active transport of glucose and Na^+ induces water influx in the small intestine rather than that glucose is mainly transferred paracellulary by solvent drag. This corrresponds to the recently proposed hypothesis that water transport is directly linked to solute transport by cotransport proteins such as the brush border Na^+ glucose cotransporter (Loo et al. 1996).

Thus, the results of the present investigation show that:

1) a close coupling of disaccharide hydrolysis with derived glucose absorption in chronic experiments is achieved mainly due to a high activity of glucose transporters, which are presumably not associated with membrane disaccharidases;

2) the transcellular active transport (rather than the paracellular one) may be regarded as a predominant mechanism of disaccharide-derived glucose absorption under conditions close to physiological

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