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Differential In Vivo and In Vitro Intestinal Permeability to Lactulose and Mannitol in Animals and Humans: A Hypothesis

PIETER B. BIJLSMA,* ROGER A. PEETERS,[†] JACK A. GROOT,[§] PIETER R. DEKKER,[‡]
JAN A. J. M. TAMINIAU,* and ROELOF VAN DER MEER[†]

*Department of Pediatric Gastroenterology and Nutrition, Academic Medical Center, Amsterdam; [†]Department of Nutrition, Netherlands Institute for Dairy Research, Ede; and [§]Institute of Neurobiology, University of Amsterdam, Amsterdam, The Netherlands

Background/Aims: Clinical interpretation of urinary recovery ratios of lactulose and mannitol is hampered by incomplete understanding of the mechanisms of transmucosal passage. The aim of this study was to compare in vivo and in vitro probe permeability. **Methods:** Stripped sheets of small intestine from rodents and human biopsy specimens were mounted in Ussing chambers, and mucosa-to-serosa fluxes of lactulose and mannitol were determined. Urinary recovery of orally applied probes was measured in rodents, cats, and humans. **Results:** In vitro lactulose/mannitol flux ratios were close to 0.8 in all species. Urinary recovery ratios differed between rodents and cats or humans; low ratios in cats and humans were due to high mannitol recovery. **Conclusions:** Interspecies variation in urinary recovery of mannitol is caused by differences specific for the intact small intestines in vivo. Because hyperosmolality of villus tips in vivo varies, being highest in humans and cats as a result of vascular counter-current multiplication, it is hypothesized that the high urinary recovery of mannitol in these species is caused by solvent drag through pores that allow the passage of mannitol but not of lactulose. Therefore, the lactulose/mannitol ratio is primarily a standard for the normal functioning of villus epithelial cells in metabolite absorption and for normal villus blood flow.

The urinary recovery ratio of orally ingested lactulose and mannitol is frequently used as a noninvasive tool in the diagnosis of intestinal disorders. An increased ratio is usually considered to be indicative of changes in small intestinal barrier function and/or morphology, e.g., an increased permeability to antigenic luminal substances and villus atrophy. Mean mannitol recoveries in humans range from 16% to 20%, whereas the lactulose recoveries range from 0.2% to 0.8%, an unexpectedly large difference regarding the molecular dimensions of these probes. The low lactulose/mannitol (L/M) recovery ratio (about 0.025) is caused neither by a difference in luminal processing in the small intestine nor large differences in

metabolism or renal clearance of these two compounds.¹ Diffusion of lactulose and mannitol through nonrestrictive aqueous pores follows diffusion of solutes in aqueous solution, which is inversely related to the square root of their molecular weight or to the cube root of their molecular weight for solutes with a molecular weight larger than ± 15 times the molecular weight of the solvent.² Resulting L/M diffusion ratios would be 0.73 or 0.81, respectively. Application of the Stokes–Einstein diffusion relation, in which diffusion is inversely related to the molecular radius of the solute, leads to comparable L/M diffusion ratios: 0.71 to 0.80, based on mannitol and lactulose diameters of 0.67 and 0.95 nm³ or 0.8 and 1.0 nm, respectively.⁴

The discrepancy between L/M urinary recovery ratios and theoretical L/M diffusion ratios led to the concept of the intestinal epithelium as a heteroporous layer with a high incidence of small pores permitting the diffusion of mannitol while excluding the passage of lactulose and a small population of larger pores allowing the diffusion of both lactulose and mannitol. It is generally accepted that the larger pores are located in the tight junctions. Two hypotheses about the location of the small pores and, hence, the main mannitol diffusion pathway have been described: the transcellular model formulated by Menzies⁴ and the paracellular model formulated by Hollander.⁵ The transcellular model postulates that “permeation of water-soluble, lipid-insoluble molecules of mannitol-like radius or below can take place freely through numerous small ‘water pores’ situated in the cell membranes of the mucosal enterocytes, whereas those of greater size can only pass very slowly through large paracellular ‘water channels’ of low incidence.” The paracellular model is based on the difference in tight junction

Abbreviations used in this paper: HPLC, high-pressure liquid chromatography; HRP, horseradish peroxidase; L/M, lactulose/mannitol (ratio).

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structure in villi and crypts and on a difference in accessibility of luminal compounds to villi and crypts. The villus tight junctions have more strand structures and are postulated to be selectively permeable to mannitol-sized and smaller solutes, whereas the crypt tight junctions have fewer strands and are permeable to both lactulose and mannitol but are less accessible for luminal substances than villus tight junctions. Both Menzies¹ and Hollander⁶ stress that experimental proof for their hypothesis is scarce and that the basic mechanisms regulating the *in vivo* intestinal permeation of probes of differing sizes are incompletely understood.

Interestingly, the L/M urinary recovery ratios in rodents may exceed the values in humans by a factor 10 or more.⁷ There is no comprehensive explanation for this difference.

In intestinal research *in vitro*, mannitol is used as a probe for paracellular permeability,⁸ but, to our knowledge, there are no studies comparing the simultaneous *in vitro* intestinal permeability of mannitol and lactulose in humans or laboratory animals.

The question arises whether the large difference between mannitol and lactulose recovery in humans *in vivo* also applies to the human intestine *in vitro* and to laboratory animals either *in vivo* or *in vitro*. Therefore, we studied the permeability of mannitol, lactulose, and horseradish peroxidase (HRP) in stripped sheets of ileum of rats, guinea pigs, and rabbits and in human small intestinal biopsy specimens mounted in Ussing chambers. We also determined the *in vitro* mucosa-to-serosa flux of polyethylene glycol (PEG) 4000 and investigated the effect of solvent flow on the [⁵¹Cr]ethylenediaminetetraacetic acid (EDTA)/mannitol ratio in stripped rat ileum. Furthermore, we examined the urinary recovery of lactulose and mannitol after oral ingestion in rats, guinea pigs, rabbits, cats, and humans.

Materials and Methods

In Vitro Experiments

Female rats (Wistar; 250–350 g), guinea pigs (Dunkin Hartley; 350–600 g), and rabbits (New Zealand white; 1.5–2.5 kg) were anesthetized by intraperitoneal injection (rats, 1.0 mL/kg) or killed by intraperitoneal (guinea pigs, 1.5 mL/kg) or intravenous (rabbits, 1.0 mL/kg) injection of Nembutal (Abbott, Santa Clara, CA). In rats, a midline abdominal ventral incision was made, and segments of distal ileum were ligated, incised next to the ligatures, and rinsed with Ringer's solution to remove intestinal contents. After ligating the blood supply to the segment, it was removed and rapidly placed in an ice-cold, carbogenated (humidified 95% O₂ plus 5% CO₂) Krebs–Ringer's solution. The segment was stripped of muscle layers, and flat sheets of tissue were mounted in

Ussing chambers within 5 minutes after cutoff of the blood supply. In rabbits and guinea pigs, a ventral abdominal incision was made, and the ileum was rapidly removed, rinsed of luminal contents, and placed in an ice-cold, carbogenated Krebs–Ringer's solution. A distal segment was stripped of muscle layers, and tissues were mounted in Ussing chambers within 10 minutes after the death of the animal. Exposed serosal area was 0.2 cm² (rat, guinea pig) or 3.14 cm² (rabbit), free of Peyer's patches. Silicone grease was used to minimize edge damage. Both compartments contained 3 mL (rabbits, 10 mL) of a thermostated (37°C), carbogenated, and continuously stirred Krebs–Ringer's bicarbonate solution. Ringer's composition was (in mmol/L) as follows: NaCl, 117.5; KCl, 5.7; NaHCO₃, 25.0; NaH₂PO₄, 1.2; CaCl₂, 2.5; and MgSO₄, 1.2, with a pH of 7.3 after carbogenation and osmolality of 290 mOsm/kg (after addition of probe solutions). In some experiments, transepithelial potential difference and resistance were continuously monitored with Ringer-calomel electrodes and by voltage deflections induced by 10- μ A bipolar current pulses through platinum wires. Resistance was calculated according to Ohm's law. The electrodes and platinum wires were connected with the chambers via Ringer-agar bridges.

Human forceps biopsy specimens were obtained by routine oral endoscopy (duodenum) or colonoscopy (ileum) and immediately placed in an ice-cold carbogenated Krebs–Ringer's solution. Suitable tissue was orientated and mounted under a dissection microscope in Ussing chambers as used by Grasset et al.,⁹ using a modified aperture. The time between obtaining the specimens and the mounting in Ussing chambers was <15 minutes. Compartments contained 1.5 mL Ringer's; exposed serosal area was 0.0175 cm²; and transepithelial potential difference and resistance were continuously monitored as described earlier using 1- μ A bipolar current pulses through platinum wires connected to the chambers via Ringer-agar bridges.

In the first series of experiments with rat ileum, after a 15-minute equilibration period, 10 μ mol/L [¹⁴C]mannitol (2 μ Ci), 10 μ mol/L [³H]PEG 4000 (10 μ Ci), and 10 μ mol/L HRP were added to the mucosal compartment (final osmolality, 290 mOsm/kg). Serosal samples were taken every 15 minutes and replaced by an equal volume. In the next series, 10 mmol/L of both mannitol and lactulose were added to the mucosal side (in some of the experiments, together with 10 μ mol/L HRP), and 10 mmol/L inosine and 5 mmol/L NaCl were added to the serosal side to maintain isosmolality. Serosal samples of 0.2 mL were taken every 15 minutes during 150 minutes and replaced by fresh Ringer's solution containing 10 mmol/L inosine and 122.5 mmol/L NaCl. The samples were analyzed using high-performance liquid chromatography (HPLC). In other experiments, 50 μ Ci [⁵¹Cr]EDTA and 10 μ Ci [¹⁴C]mannitol were added together with 10 mmol/L mannitol and lactulose to the mucosal side, and serosal samples of 0.2 mL were taken and replaced by fresh Ringer's every 4 minutes during the first 20 minutes, at 30 minutes, and every 15 minutes during the remaining 120 minutes. In some of these experiments (rat ileum only), the mucosal bathing solution

was replaced by a hypotonic Ringer's solution (134 mOsm/kg) with reduced NaCl content or the serosal solution was replaced by a hypertonic Ringer's solution (640 mOsm/kg) containing a higher mannitol concentration at the time of probe additions.

HPLC

Serosal samples of 100 μ L (containing 0.002% NaN_3 as a preservative) were analyzed for mannitol and lactulose content by HPLC using two anion exchange columns in series (Carbonpack PA1; Dionex, Breda, The Netherlands), with a 0.1-mol/L NaOH solution as the isocratic mobile phase followed by pulsed amperometric electrochemical detection on a gold electrode (Dionex PED).

Detection of Radiochemicals

Samples of 100 or 200 μ L were mixed with 5 mL scintillation fluid (Lumogold; Packard, Meriden, CT) and counted in a scintillation counter (Packard 1600CA) using the ^3H window for detection of [^3H]EDTA. Correlation coefficient between radiochemical and HPLC detection of mannitol was >0.99 .

Colorimetric Assay of HRP Activity

The appearance of intact HRP in the serosal bath was measured enzymatically. In short, a 0.1-mL sample of test solution was mixed with 1.4 mL phosphate buffer (0.1 mol/L, pH 6.0) containing 0.003% H_2O_2 and 0.009% ortho-dianisidine dihydrochloride. The linear, HRP concentration-dependent rate of increase in optical absorption at 460 nm was determined with an Eppendorf photometer (Hamburg, Germany).

In Vivo Experiments

Animals were placed in metabolic cages at 6 AM with free access to water and food, and control urine was collected until 6 PM. At 6 PM, an aqueous solution containing 0.1 g/mL mannitol and 0.2 g/mL lactulose (1140 mOsm/kg) was given intrapharyngeally with a cannulated syringe (rabbits and cats, 5.0 mL; guinea pigs and rats, 1.0 mL). Urine was collected overnight until 6 AM, and NaN_3 (0.002%) was added as a preservative. During the experiments, food intake by the animals was minimal, resembling the mild fasting conditions in the human tests. Volunteers fasted during the morning until 12 AM but were allowed to drink water or tea without sugar; at 9 AM, after collection of control urine, they ingested a 100-mL aqueous solution containing 2.3 g mannitol and 5.5 g lactulose (290 mOsm/kg). One volunteer was intubated with a flexible polyethylene tube inserted into the small intestine until the ligament of Treitz under X-ray monitor guidance; 200 mL of an isosmolar test solution containing 4.6 g mannitol and 11.0 g lactulose was continuously infused with a volumetric pump (Ivac 591 Star Flow; Ivac, Nieuwegein, The Netherlands) during 1.5 hours (flow rate, 2.2 mL/min). Complete

urine collections were made for 6 hours until 3 PM. NaN_3 (0.002%) was added as a preservative.

Urine Preparation for HPLC

A saturated NaOH solution was added (10 μ L/ml urine), urine was stirred for 10 seconds and centrifugated for 10 minutes (2500 rpm), and the supernatant was collected. Then 0.5 g of Duolite MB 5113 resin was added per milliliter of supernatant, stirred for 1 hour, and precipitated. Samples from animals were diluted 10-fold and human samples were diluted 50-fold in distilled water before HPLC analysis of 100- μ L aliquots as described earlier.

Ethical Guidelines

The *in vitro* studies of human intestinal tissue were approved by the local medical ethical committee, and all animal studies were approved by the local welfare committee for animal experiments.

Results

In Vitro Experiments

Electrophysiology. All tested tissues except those from guinea pigs showed a serosa-positive potential difference of 1–3 mV after mounting in the Ussing chambers; this difference decreased to steady-state values of 0.2–1.5 mV within 15–30 minutes. Transepithelial resistance at this time ranged from 20 to 40 $\Omega \cdot \text{cm}^2$ and remained constant during the time course of the flux experiments (150 minutes from time of addition of the probes). Guinea pig ileum had higher steady-state potential difference values (about 4 mV) and resistance values of 40–50 $\Omega \cdot \text{cm}^2$. Histological appearance of tissues at the end of experiments judged by light and electron microscopy was normal, except for excess mucus production, especially by rabbit and human tissues. The maintenance of a low cellular electrochemical sodium potential by the tissues was tested by bilateral addition of 10 mmol/L D-glucose at the end of the flux experiments, resulting in serosa-positive potential changes of about 2 mV in human specimens and 4–6 mV in the animal tissues. This shows that even after prolonged experimental periods, the cells maintain their sodium gradient; therefore, apparently they are not anoxic.

Mannitol, PEG 4000, and HRP flux. In the first series of experiments, we compared the fluxes of the probes, [^{14}C]mannitol, [^3H]PEG 4000 (average molecular weight, 4 kilodaltons), and HRP (molecular weight, 40 kilodaltons), through stripped rat ileum ($n = 7$). The fluxes of mannitol and PEG 4000 reached a steady-state value at 30 minutes and remained constant until the end of the experimental period (150 minutes). Mannitol flux was $184 \pm 30 \text{ pmol} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$, and PEG 4000 flux was

Table 1. In Vitro Steady-State Fluxes of Lactulose and Mannitol and L/M Flux Ratios

Species and tissue	Mannitol flux ($\text{nmol} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$)	Lactulose flux ($\text{nmol} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$)	L/M flux ratio	n
Guinea pig ileum	133 \pm 10	100 \pm 3	0.76 \pm 0.04	6
Rat ileum	174 \pm 24	118 \pm 15	0.68 \pm 0.02	8
Rabbit ileum	70 \pm 11	62 \pm 9	0.89 \pm 0.03	4
Human duodenum	153 \pm 13	127 \pm 14	0.84 \pm 0.09	4
Human ileum	193 \pm 30	144 \pm 16	0.78 \pm 0.05	8

NOTE. Fluxes are means \pm SEM from 30 to 150 minutes; n = number of tissue preparations per biopsy specimen (two tissues per animal or human).

27 \pm 5 $\text{pmol} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$. The flux of HRP reached a steady-state value at about 75 minutes (3.1 \pm 0.4 $\text{pmol} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$) and remained constant. The apparent permeability coefficients for mannitol and PEG 4000 were calculated from the flux divided by the concentration as follows: $P_{\text{mannitol}} = 5.1 \cdot 10^{-6} \pm 0.8 \cdot 10^{-6} \text{ cm/s}$ and $P_{\text{PEG 4000}} = 0.75 \cdot 10^{-6} \pm 0.14 \cdot 10^{-6} \text{ cm/s}$. Their ratio was 0.15 \pm 0.02. The HRP fluxes in the other tissues were comparable with the flux in rat ileum (guinea pig ileum, 2.5 \pm 0.5 [n = 5]; rabbit ileum, 4.3 \pm 1.8 [n = 6]; human duodenum, 4.5 \pm 0.8 [n = 4]; and human ileum, 3.1 \pm 1.1 $\text{pmol} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$ [n = 8]).

Mannitol and lactulose flux. Steady-state fluxes of both mannitol and lactulose from 30 to 150 minutes and flux ratios during the same time span are summarized in Table 1. In all preparations, mean fluxes of lactulose and mannitol were in the range of 100 $\text{nmol} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$, and L/M flux ratios were close to theoretical diffusion ratios through nonrestrictive aqueous pores (0.7–0.8).

To compare the time course of the fluxes during the first 30 minutes after addition of the probes, we used [^{14}C]mannitol and, instead of lactulose, [^{51}Cr]EDTA, because HPLC detection of lactulose and mannitol during this initial period was not sensitive enough to detect flux kinetics during short-term intervals (4 minutes).

Results of human duodenal biopsy specimens are summarized in Figure 1. The flux data for both probes show similar kinetics. Moreover, the [^{51}Cr]EDTA/[^{14}C]mannitol flux-ratios were comparable with L/M ratios as detected by HPLC (Table 1). Absolute and relative data similar to those obtained for human specimens in Figure 1 were obtained with rabbit, rat, and guinea pig ileum (results not shown).

Effect of anisomotic solutions. In an attempt to manipulate the solute flux ratio through the stripped rat ileum, we used anisomotic solutions and measured the [^{14}C]mannitol and [^{51}Cr]EDTA fluxes. Control [^{51}Cr]EDTA/[^{14}C]mannitol flux ratio was 0.78 \pm 0.01 (n = 8). With serosal hyperosmolar Ringer's solution, the individual fluxes increased but the flux ratio decreased to 0.59 \pm 0.04 (n = 4; $P < 0.01$). With mucosal

hyposmolar solution, the flux ratio decreased to 0.66 \pm 0.03 (n = 5; $P < 0.01$), whereas the individual fluxes decreased 2–3-fold. Bilateral hyposmolality of 134 mOsm/kg gave a similar decrease of probe fluxes, but [^{51}Cr]EDTA/[^{14}C]mannitol flux ratio was 0.80 \pm 0.01 (n = 4), not different from control values.

In Vivo Experiments

The urinary recoveries of lactulose and mannitol, expressed as a percentage of oral dose, and the L/M ratios found in the tested species are summarized in Table 2. L/M ratios in guinea pigs and rats were close to in vitro L/M flux ratios (Table 1) or differed by a factor 3 in rabbits. In contrast, this difference exceeds a factor 30 in humans, because of an extremely high mannitol recovery.

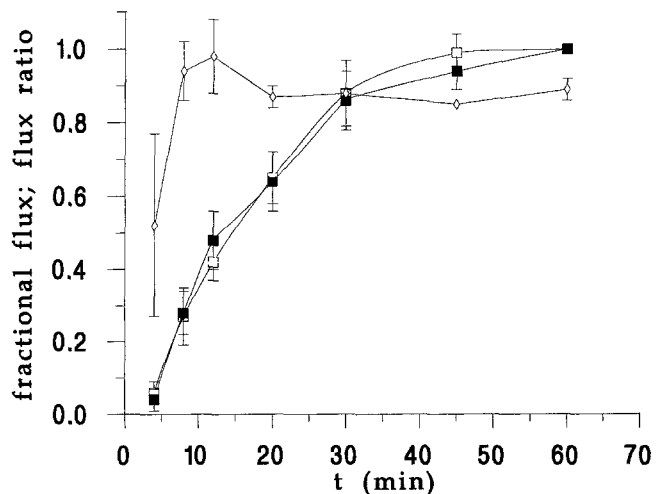


Figure 1. Time course of development of steady-state mucosal-to-serosal [^{51}Cr]EDTA (■) and mannitol (□) fluxes and [^{51}Cr]EDTA/mannitol flux ratios (◇) in human duodenum in vitro. Because of the difference in concentrations of [^{51}Cr]EDTA (0.1 mmol/L) and mannitol (10 mmol/L), fluxes are presented as a fraction of the steady-state flux (steady-state mannitol flux from 45 to 150 minutes, 244 \pm 14 $\text{nmol} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$; steady-state [^{51}Cr]EDTA flux, 2.10 \pm 0.14 $\text{nmol} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$). [^{51}Cr]EDTA/[^{14}C]mannitol flux ratios were corrected for the difference in concentration. All data points represent mean \pm SEM (n = 3). Because of low counts, the first flux ratio at 4 minutes is unreliable.

Table 2. In Vivo Urinary Recoveries of Lactulose and Mannitol and L/M Recovery Ratios

Species	Mannitol recovery (% of applied dose)	Lactulose recovery (% of applied dose)	L/M recovery ratio	n
Guinea pig	4.95 ± 1.65	3.00 ± 1.10	0.60 ± 0.02	2
Rat	4.05 ± 1.48	2.07 ± 0.66	0.56 ± 0.06	4
Rabbit	2.12 ± 0.74	0.64 ± 0.27	0.28 ± 0.06	5
Human	21.5 ± 2.64	0.47 ± 0.09	0.022 ± 0.002	5
Cat	28.5 ± 3.25	0.88 ± 0.16	0.030 ± 0.002	2

NOTE. The urinary recoveries of lactulose and mannitol are expressed as percentage of applied dose. Recovery time in animals was 12 hours in animals and 6 hours in humans; n = number of animals or human volunteers. Values are mean ± SEM.

Urinary recoveries and L/M ratios of cats closely resembled those of humans.

In animal studies, hyperosmolar solutions were given, whereas humans ingested isotonic solutions. After application of hyperosmolar test solutions to humans (5 g mannitol and 10 g lactulose in 50 mL H₂O; 1140 mOsm/kg), urinary recovery of mannitol was 17.5% ± 0.5%, lactulose recovery was 0.33% ± 0.08%, and L/M ratio was 0.019 ± 0.004 (n = 2). These values are comparable with those obtained with isosmolar test solutions.

To compare L/M recovery ratios of in vivo bolus ingestion with those of in vivo continuous infusion in humans, a single in vivo experiment with continuous infusion of lactulose and mannitol was performed. Six-hour urinary recovery of mannitol was 10.8% and that of lactulose 0.35%, giving an L/M ratio of 0.033. After isosmolar bolus ingestion by the same volunteer, 6-hour mannitol recovery was 14.5%, lactulose recovery was 0.33%, and L/M ratio was 0.023, indicating no marked difference in recovery between bolus and continuous application of probe solutions in humans.

Finally, as a preliminary test for the hypothesis formulated in the Discussion, 8 volunteers ingested 100 mL of an isosmolar test solution containing 27.4 mmol/L of both lactulose and mannitol, 66.1 mmol/L NaCl, and 100 mmol/L glucose (290 mOsm/kg). Six-hour urinary recovery was 35.9% ± 4.3% for mannitol and 0.58% ± 0.11% for lactulose, and L/M ratio was 0.016 ± 0.002. Mannitol recovery was significantly higher and L/M ratio was significantly lower than values shown in Table 2 ($P < 0.01$ and $P < 0.05$, respectively; one-tailed *t* test).

Discussion

The most noticeable finding in this study and the study of Delahunty and Hollander⁷ is that the high urinary mannitol recovery of humans and cats (Table 2) was not observed in rats, guinea pigs, and rabbits and was not reflected in a high mannitol permeability in in vitro experiments (Table 1). It is suggested that the high mannitol recovery in humans is caused by the diffusion through a large number of small pores that exclude lactu-

lose. It may be postulated that rodents do not have these small pores; however, we hypothesize that rodents have these pores but that their presence is less manifest in vivo than in humans and cats and that diffusion through these pores is even less evident in in vitro experiments.

The second finding is that in vitro human tissue does not show the large discrimination between mannitol- and lactulose-sized molecules. It may be suggested that the in vitro experiments with human tissue do not reflect the in vivo situation because the excised tissue has acquired artifactual large pores that pass lactulose without restriction. Consequently, based on the high mannitol recovery in vivo, we might expect to find a high lactulose permeability in vitro in case of tissue damage. However, a comparison of the in vitro mannitol fluxes in rodents and humans and the lactulose fluxes does not corroborate this suggestion. A more appropriate interpretation of the in vitro fluxes may be that the human intestinal tissues lose their high mannitol permeation after excision (see item 2 below).

Permeability Studies In Vitro

We do not know of a method to measure the passive permeability coefficient of in vivo epithelium without complicating factors of absorptive and secretory water flow and effects of blood flow. However, the results show arguments to validate in vitro experiments in permeability studies.

1. The HRP flux values were similar to values reported previously¹⁰ and appeared to result primarily from endocytosis (Kiliaan, Bijlsma, Scholten, Taminau, and Groot, unpublished observations, and Walker et al.¹¹). The permeability ratio of PEG 4000 to mannitol (0.15 ± 0.02) is less than expected from free diffusion (0.21 or 0.36, based on square or cube ratios of molecular weights), suggesting some restriction.
2. The in vitro studies (Table 1) show the interspecies similarity of lactulose and mannitol permeability. There is no evidence for an increased lactulose flux in human tissue, as would have been expected if the

experimental handling of this tissue had induced larger pores. Instead, the *in vitro* mannitol flux data are not different from the data of rodents, therefore suggesting that the mannitol flux is smaller than *in vivo*.

3. From the flux ratios of PEG 4000 to mannitol (0.15 ± 0.02) and lactulose to mannitol (0.68 ± 0.02) observed in *in vitro* rat ileum, we can calculate the PEG 4000/lactulose flux ratio (0.22). This is comparable with the urinary recovery ratio of probes of similar molecular diameter (fluorescein isothiocyanate-dextran 3000/lactulose, 0.19) obtained in *in vivo* experiments with humans.¹² This shows the similarity of rat intestine *in vitro* and human intestine *in vivo* with respect to the permeability of this range of larger molecules.
4. The *in vitro* permeability found for mannitol in the present study is fully comparable with recently published values for *in vitro* rat ileum stripped from its serosal layer¹³ and is only 1.3 times larger than found with nonstripped ileum.¹⁴
5. The reduction of the *in vitro* [⁵¹Cr]EDTA/mannitol flux ratio through rat ileum by anisosmotic solutions shows that, under special circumstances, the permeation of mannitol can be preferentially increased, with lactulose being restricted.
6. The observed L/M and [⁵¹Cr]EDTA/mannitol ratios are in the same range as [⁵¹Cr]EDTA/mannitol ratios measured in monolayers of the rat IEC-18 ileal cell line (mean value, 0.67¹⁵), which has a transepithelial resistance of about 30 $\Omega \cdot \text{cm}^2$, comparable with the values for animal and human tissues. The filter-grown cells do not require extensive dissection and handling but apparently have no mannitol-selective pores.
7. The electrophysiological parameters of the *in vitro* small intestine of rats, rabbits, and guinea pigs in our study are all close to literature values,^{16–18} and these parameters and glucose-elicited potential responses in the human biopsy specimens are similar to those reported for jejunal suction biopsy specimens.¹⁹

Therefore, we suggest that the observed *in vitro* L/M ratios in rodent small intestinal tissues, which are close to theoretical diffusion ratios of these probes through nonrestrictive aqueous pores, reflect the *in vivo* permeability ratio in these species and that the human tissue has lost the high mannitol permeation after excision. Also, the kinetics of [⁵¹Cr]EDTA and mannitol fluxes through *in vitro* human duodenum (Figure 1) suggest that both probes diffuse with the same time course through a similar pathway and that the expected high-permeability pathway for mannitol is not apparent.

Comparison of In Vivo Animal and Human Recoveries

A potentially complicating factor in the comparison of the *in vivo* data is the difference in recovery time between animal (12 hours, due to the time required to collect urine from the cats and rabbits) and human experiments (6 hours). However, according to the literature,^{20–22} the 0–24-hour and 0–10-hour recovery ratios are similar to the 0–5-hour recovery ratio in humans. Thus, we feel confident in comparing the 0–6-hour recoveries in humans with the 0–12-hour recoveries in animals. Another complicating factor, namely, the difference in osmolality of test solutions applied to animals and humans, can also be ruled out by human test results: L/M recovery ratios in human volunteers after hyperosmolar (1140 mOsm/kg) or isosmolar test solutions were in the same range, corroborating reports^{4,22} that lactulose and *l*-rhamnose recoveries in humans show only minor changes until the test solution osmolality is increased to 1500 mOsm/kg or higher.

The question remains what pathway is used by mannitol *in vivo* so that the recovery in humans and cats is about an order of magnitude larger than in the rodents (Table 2) and why this high mannitol permeation is lost in humans after excision. In the following section, we address the relevance of factors differing between rodents and humans or cats.

Villus Heights

Considering clinical data of double sugar tests in the diagnosis of small intestinal diseases, undisputable increases in L/M ratios are obtained in cases of celiac disease, in which mannitol recovery is decreased and lactulose recovery is enhanced. These recovery changes are most prominent when there is a marked villus atrophy.^{23–25} Because the species in our study show substantial differences in villus height, the question arises whether the villus height correlates with L/M ratios. We averaged relevant literature data^{26–52} on villus height in Table 3. Comparison of these villus height data to *in vivo* L/M ratios shows an inverse correlation only in the case of ileal villus height values. In contrast, there is no such correlation between villus height and L/M ratios *in vitro*. Thus, it can be concluded that this morphological difference alone cannot explain the interspecies variation in L/M ratios. Accordingly, the difference between *in vitro* and *in vivo* L/M ratios and the interspecies difference in permeability ratio *in vivo* should be caused by physiological factors unique for the intact intestine in living animals and humans.

Table 3. Summary of Literature Data of Villus Heights in Micrometers

Species	Duodenum	n (N)	Jejunum	n (N)	Ileum	n (N)	Study (ref no.)
Guinea pig	—	— (0)	576 ± 20	15 (1)	199 ± 8	11 (1)	26, 27
Rat	573 ± 48	26 (3)	415 ± 54	48 (8)	272 ± 44	36 (5)	28–37
Rabbit	1010 ± 5	120 (1)	754 ± 112	28 (4)	553 ± 34	13 (2)	38–42
Human	636 ± 41	30 (3)	640 ± 38	40 (2)	567 ± 39	24 (2)	43–49
Cat	1072	6 (1)	963	2 (1)	760 ± 20	28 (1)	50–52

NOTE. Values are mean ± SEM using relative error analysis in case of multiple sources to obtain overall SEM. n, number of animals or humans; N, number of references. Values of duodenum and jejunum in cats represent medians as given in the references.

Villus Hyperosmolality

One of the main differences between the *in vitro* and *in vivo* situation is the blood supply. An important consequence of the opposite blood flow in villus arterioles and capillaries is the maintenance of hyperosmolality in the villus tips as a result of countercurrent multiplication.^{53–56} Because of interspecies variation in architecture of the villus blood vessels,⁵⁷ there is a varying efficiency of the countercurrent exchange, leading to differences in villus tip hyperosmolality. The comparison of these tip osmolalities, summarized in a study of Hallbäck et al.,⁵⁸ to *in vivo* L/M ratios also shows a negative correlation (Figure 2). The interspecies similarity in *in vitro* L/M ratios and in *in vitro* villus tip osmolalities due to the absence of a countercurrent mechanism is consistent with this correlation. It should be noted that Hallbäck et al.⁵⁵ argued that their data of the villus hyperosmolality in *in vivo* human small intestine may be an underestimation

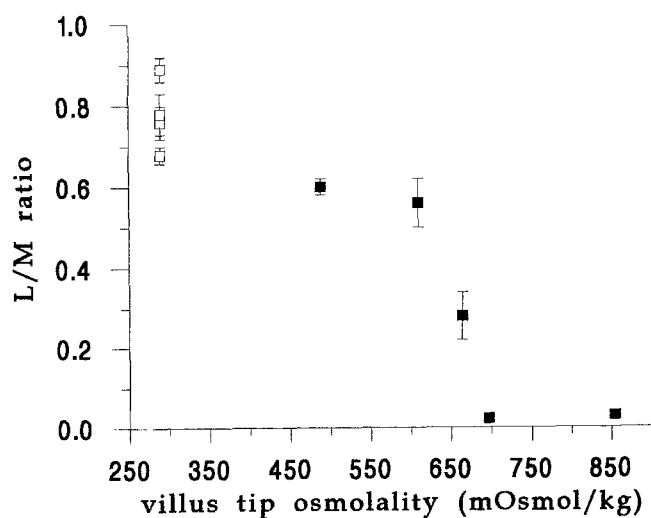


Figure 2. Plot of *in vitro* L/M flux ratios (□) (from Table 1) and *in vivo* L/M urinary recovery ratios (■) (from Table 2) against villus tip hyperosmolality values. *In vitro* tip osmolalities are 290 mOsm/kg. *In vivo* tip osmolalities are mean values for small intestine from Hallbäck et al.,⁵⁴ being 489 mOsm/kg for guinea pigs, 610 for rats, 665 for rabbits, 697 for humans, and 855 for cats.

of the actual physiological values because of methodological limitations that were not present in their animal studies.

Because a high villus osmolality induces water absorption, we suggest that the difference between lactulose and mannitol recovery *in vivo* in humans and cats is caused by the solvent drag of mannitol induced by hyperosmolality in their villus tips. The solvent drag may only affect the flux of mannitol, presumably because the solvent flux is through pores that selectively allow the passage of mannitol and/or smaller molecules and are restrictive to lactulose-sized substances. As a consequence of the small difference in molecular diameter of lactulose and mannitol, the mannitol passage through these pores must occur in a single-file array, which may explain the small contribution of these pores to the *in vitro* passive diffusion of mannitol when no solvent flow drags the molecules through these small pores. This hypothesis allows for a combined explanation of the *in vivo* and *in vitro* small intestinal permeation of both probes in various species. An advantage of this solvent drag-related model to existing transcellular and/or paracellular passive diffusion-related hypotheses is that it permits experimental testing and validation with existing techniques. For instance, as shown in Results, we induced solvent drag across stripped rat ileum in an Ussing chamber by a mucosa-to-serosa osmotic gradient and found that the mucosa-to-serosa water flow could decrease the [⁵¹Cr]-EDTA/mannitol ratio. This shows that also in stripped rat ileum, there is some evidence for the existence of small mannitol-selective pores. With this type of experiment, hyperosmolality is not restricted to the villi and solvent drag may also occur through the crypt regions.

One may affect the hyperosmolality in the villus tip by modulation of the apical Na⁺-coupled glucose carrier.⁵³ The hypothesis predicts that in the presence of luminal glucose and NaCl, the solvent drag of mannitol should increase. Recently, Jodal et al.⁵⁹ using *in situ* perfusion of the lumen of rat jejunum with a Krebs-Henseleit solution containing [⁵¹Cr]EDTA and [¹⁴C]-

mannitol found that the [^{51}Cr]EDTA/mannitol clearance ratio was 0.47. This ratio is an order of magnitude larger than the urinary recovery ratio in humans and cats and of the same magnitude as found *in vitro*. Interestingly, this ratio decreased by a factor 3 in the presence of 30 mmol/L glucose in the isosmolar perfusate, as a result of a threefold increase of the mannitol clearance. In our study with human volunteers, the urinary recovery of mannitol increased with a factor 1.7 and L/M ratio decreased with a factor 1.4 after oral ingestion of a test solution containing glucose plus NaCl, whereas the lactulose recovery was not significantly different from values obtained with test solutions containing only lactulose and mannitol. These results suggest a selective coupling of mannitol fluxes to enhanced water absorption induced by the active uptake of Na^+ and glucose through villus enterocytes. Moreover, the clinical observation of a decreased mannitol recovery in the case of villus atrophy, as seen in celiac disease,^{23–25} is readily explained by an impairment in the efficiency of the countercurrent multiplication mechanism caused by shortening of the vascular structure in the villi. Similar observations have been reported with gluten-sensitive dogs.⁶⁰ The cellobiose/mannitol recovery ratio, which is 0.05 during gluten-free diet, increased significantly during a wheat-containing diet because of reduction of mannitol recovery. Before gluten challenge, both control dogs and sensitive dogs showed a 5-hour urinary recovery of mannitol of about 50%, which correlates well to the finding of Bond et al.⁵⁶ of a very efficient countercurrent multiplication mechanism in this species. The efficiency of this mechanism will also be reduced in states of both intestinal ischemia and excessive, nonabsorptive hyperemia.^{53,61} Assuming that *in vivo* mannitol recovery predominantly reflects the magnitude of solvent drag caused by villus tip hyperosmolality, individual recovery data of mannitol and lactulose may provide more specific clinical information than only L/M ratios. Whereas lactulose recovery may represent paracellular passive diffusion over the mucosal barrier as a whole, mannitol recovery depends mainly on water absorption in the upper part of the villus. In general, as a consequence of the “solvent drag hypothesis,” mannitol recovery will be reduced by malabsorption of sodium and/or impaired sodium-coupled nutrient uptake, by disturbances in blood flow through the villi, or by shortening of the villi and their vasculature.

Recently, two reports^{62,63} showing evidence for solvent drag as one of the mechanisms for mannitol uptake from *in vivo*—perfused rat intestine were published. Although lactulose-sized probe permeability was not determined in a study by Krugliak et al.,⁶² their finding of a reduction of

water absorption and the solvent drag—mediated component of mannitol uptake by luminal hyperosmolar solutions and by the secretory agent chenodeoxycholic acid stresses the importance of this mechanism. In addition, the finding of Jodal et al.⁶³ that blocking of neurally mediated cholinergic secretory activity by atropine and hexamethonium caused increased water and mannitol absorption, whereas [^{51}Cr]EDTA uptake was unaffected, indicates the restriction of solvent drag effects to mannitol-sized (and smaller) probe molecules.

References

1. Travis S, Menzies I. Intestinal permeability: functional assessment and significance. *Clin Sci* 1992;82:471–488.
2. Stein WD. The movement of molecules across cell membranes. New York: Academic, 1967:65–126.
3. Hollander D, Ricketts D, Boyd CAR. Importance of “probe” molecular geometry in determining intestinal permeability. *Can J Gastroenterol* 1988;2:35A–38A.
4. Menzies IS. Transmucosal passage of inert molecules in health and disease. In: Skadhauge E, Heinze K, eds. Intestinal absorption and secretion. Falk Symposium 36. Lancaster, England: MTP, 1984:527–543.
5. Hollander D. The intestinal permeability barrier. A hypothesis as to its regulation and involvement in Crohn’s Disease. *Scand J Gastroenterol* 1992;27:721–726.
6. Hollander D. Permeability in Crohn’s disease: altered barrier functions in healthy relatives? *Gastroenterology* 1993;104:1848–1873.
7. Delahunty T, Hollander D. A comparison of intestinal permeability between humans and three common laboratory animals. *Comp Biochem Physiol* 1987;86A:565–567.
8. Madara JL, Stafford J. Interferon-gamma directly affects barrier function of cultured intestinal epithelial monolayers. *J Clin Invest* 1989;83:724–727.
9. Grasset E, Heyman M, Dumontier AM, Lestrade H, Desjeux JF. Possible sodium and D-glucose cotransport in isolated jejunal epithelium of children. *Pediatr Res* 1979;13:1240–1246.
10. Heyman M, Ducroc R, Desjeux JF, Morgat JF. Horseradish peroxidase transport across adult rabbit jejunum *in vitro*. *Am J Physiol* 1982;242:G558–G564.
11. Walker WA, Cornell R, Davenport LM, Isselbacher KJ. Macromolecular absorption: mechanism of horseradish peroxidase uptake and transport in adult neonatal rat intestine. *J Cell Biol* 1972;54:195–205.
12. Wheeler PG, Menzies IS, Creamer B. Effect of hyperosmolar stimuli and coeliac disease on the permeability of the human gastrointestinal tract. *Clin Sci Mol Med* 1978;54:495–501.
13. Artursson P, Ungell AL, Lofroth JE. Selective paracellular permeability in two models of intestinal absorption—cultured monolayers of human intestinal epithelial cells and rat intestinal segments. *Pharmacol Res* 1993;10:1123–1129.
14. Munck BG, Rasmussen SN. Paracellular permeability of extracellular space markers across rat jejunum *in vitro*. Indication of a transepithelial fluid circuit. *J Physiol* 1977;271:473–488.
15. Ma TY, Hollander D, Bhalla D, Nguyen H, Krugliak P. IEC-18, a nontransformed small intestinal cell line for studying epithelial permeability. *J Lab Clin Med* 1992;120:329–341.
16. Crowe SE, Sodak, Stanisz AM, Perdue MH. Intestinal permeability in allergic rats: nerve involvement in antigen-induced changes. *Am J Physiol* 1993;264:G617–G623.
17. Marcon-Genty D, Tome D, Kheroua O, Dumontier AM, Heyman

- M, Desjeux JF. Transport of β -lactoglobulin across rabbit ileum in vitro. *Am J Physiol* 1989;256:G943–G948.
18. Baird AW, Cuthbert AW. Neuronal involvement in type 1 hypersensitivity reactions in gut epithelia. *Br J Pharmacol* 1987;92:647–655.
 19. Taylor CJ, Baxter PS, Hardcastle J, Hardcastle PT. Failure to induce secretion in jejunal biopsies from children with cystic fibrosis. *Gut* 1988;29:957–962.
 20. Brunetto AL, Pearson ADJ, Gibson R, Bateman DN, Rashid MU, Laker MF. The effect of pharmacological modification of gastric emptying and mouth-to-caecum transit time on the absorption of sugar probe marker molecules of intestinal permeability in normal man. *Eur J Clin Invest* 1990;20:279–284.
 21. Cobden I, Hamilton I, Rothwell J, Axon ATR. Cellobiose/mannitol test: physiological properties of probe molecules and influence of extraneous factors. *Clin Chim Acta* 1985;148:53–62.
 22. Maxton DG, Bjamason I, Reynolds AP, Catt SD, Peters TJ, Menzies IS. Lactulose ⁵¹Cr-labelled ethylenediaminetetra-acetate, l-rhamnose and polyethyleneglycol 500 as probe markers for assessment in vivo of human intestinal permeability. *Clin Sci* 1986;71:71–80.
 23. Ukabam SO, Cooper BT. Small intestine permeability to mannitol, lactulose, and polyethylene glycol 400 in celiac disease. *Dig Dis Sci* 1984;29:809–816.
 24. Nathavitharana KA, Lloyd DR, Raafat F, Brown GA, McNeish AS. Urinary mannitol: lactulose excretion ratios and jejunal mucosal structure. *Arch Dis Childhood* 1988;63:1054–1059.
 25. Juby LD, Rothwell J, Axon ATR. Cellobiose/mannitol test—a sensitive tubeless test for coeliac disease: results on 1010 unselected patients. *Gut* 1989;30:476–480.
 26. Weaver LT, Carrick BM. Changes in upper intestinal epithelial morphology and kinetics in the growing guinea pig. *Pediatr Res* 1989;26:31–33.
 27. Moore R, Carlson S, Madara JM. Villus contraction aids repair of intestinal epithelium after injury. *Am J Physiol* 1989;257:G274–G283.
 28. Debnam ES, Ebrahim HY. Autoradiographic localization of Na⁺-dependent L-valine uptake by the jejunum of streptozotocin-diabetic rats. *Eur J Clin Invest* 1990;20:61–65.
 29. Trahair JF. Remodeling of the rat small intestinal mucosa during the suckling period. *J Pediatr Gastroenterol Nutr* 1989;9:232–237.
 30. Clarke RM. The time-course of changes in mucosal architecture and epithelial cell production and cell shedding in the small intestine of the rat fed after fasting. *J Anat* 1975;120:321–327.
 31. Schroeder P, Deltz E, Seifert J, Sandforth F, Thiede A. Absorptive capacity of the transplanted small bowel. *Gut* 1987;28(Suppl 51):275–279.
 32. Nelson LM, Carmichael HA, Russel RI, Lee FD. Small-intestinal changes induced by an elemental diet (Vivonex) in normal rats. *Clin Sci Mol Med* 1978;55:509–511.
 33. Schulzke J-D, Fromm M, Bentzel CJ, Zeitl M, Menge H, Riecken E-O. Ion transport in the experimental short bowel syndrome of the rat. *Gastroenterology* 1992;102:497–504.
 34. D'Inca R, Hunt RH, Perdue MH. Mucosal damage during intestinal anaphylaxis in the rat. Effect of betamethasone and disodium cromoglycate. *Dig Dis Sci* 1992;37:1704–1708.
 35. Jonas G, Erickson RA, Morgan T. Effect of portal hypertension on in vivo bile acid-mediated small intestinal mucosal injury in the rat. *Dig Dis Sci* 1990;35:743–748.
 36. Ortega-Moreno J, Caballero-Gómez J. Uterine horn replacement in the rat using denervated and devascularized jejunum. *Eur J Obstet Gynecol Reprod Biol* 1992;45:139–145.
 37. Curtis GH, Patrick MK, Catto-Smith AG, Gall DG. Intestinal anaphylaxis in the rat. Effect of chronic antigen exposure. *Gastroenterology* 1990;98:1558–1566.
 38. Keelan M, Walker K, Thomson ABR. Intestinal morphology, marker enzymes and lipid content of brush border membranes from rabbit jejunum and ileum: effect of aging. *Mech Ageing Dev* 1985;31:49–68.
 39. Thomson ABR, Keelan M, Tavernini M, Luethe D, Lam T. Development of active and passive transport of bile acids in rabbit intestine. *Mech Ageing Dev* 1987;38:277–286.
 40. Thomson ABR. Resection of rabbit ileum: effect on jejunal structure and carrier-mediated and passive uptake. *Q J Exp Physiol* 1986;71:29–46.
 41. Thomson ABR, McIntyre Y, MacLeod J, Keelan M. Dietary fat content influences uptake of hexoses and lipids into rabbit jejunum following ileal resection. *Digestion* 1986;35:78–88.
 42. Feil W, Wenzl E, Vattay P, Starlinger M, Sogukoglu T, Schiessel R. Repair of rabbit duodenal mucosa after acid injury in vivo and in vitro. *Gastroenterology* 1987;92:1973–1986.
 43. Hasan M, Ferguson A. Measurements of intestinal villi in non-specific and ulcer-associated duodenitis—correlation between area of microdissected villus and villus epithelial cell count. *J Clin Pathol* 1981;34:1181–1186.
 44. Hasan M, Sircus W, Ferguson A. Duodenal mucosal architecture in non-specific and ulcer-associated duodenitis. *Gut* 1981;22:637–641.
 45. Schmitz J, Rey F, Bresson JL, Ricour C, Rey J. Etude par perfusion intestinale de l'absorption des sucres après résection étendue du grêle. *Arch Fr Pediatr* 1980;37:491–495.
 46. Nilsson IO, Kock NG, Lindgren I, Myrvold HE, Philipson BM, Ahren C. Morphological and histochemical changes in the mucosa of the continent ileostomy reservoir 6–10 years after its construction. *Scand J Gastroenterol* 1980;15:737–747.
 47. van der Hulst RWJ, von Meyenfeldt MF, Arends J-W, Deutz NEP, van Kreel BK, Brummer R-JM, Soeters PB. Glutamine and the preservation of gut integrity. *Lancet* 1993;341:1361–1365.
 48. Smit JM, Mulder NH, Sleijfer DT, Bouman JG, Koudstaal J, Elema JD, Veeger W. Gastrointestinal toxicity of chemotherapy and the influence of hyperalimentation. *Cancer* 1986;58:1990–1994.
 49. Fenyö G, Backman L, Hallberg D. Morphological changes of the small intestine following jejuno-ileal shunt in obese subjects. *Acta Chir Scand* 1976;142:154–159.
 50. Titkemeyer CW, Calhoun ML. A comparative study of the structure of the small intestines of domestic animals. *Am J Vet Res* 1955;16:152–157.
 51. Henry RW, Al-Bagdadi FK. Duodenal microanatomy of the domestic cat (*Felis catus*). *Histol Histopathol* 1986;1:355–362.
 52. Pilipson B. Morphology in the cat ileal mucosa following construction of an ileal reservoir or transposition of patches to different locations. *Scand J Gastroenterol* 1975;10:369–377.
 53. Haljamäe H, Jodal M, Lundgren O. Countercurrent multiplication of sodium in intestinal villi during absorption of sodium chloride. *Acta Physiol Scand* 1973;89:580–593.
 54. Jodal M. The intestinal countercurrent exchanger and its influence on intestinal absorption. In: Kramer M, Lauterbach I, eds. *Intestinal permeation*. Amsterdam: Excerpta Medica, 1977:48–55.
 55. Hallböck D-A, Hulten L, Jodal M, Lindhagen J, Lundgren O. Evidence for the existence of a countercurrent exchanger in the small intestine in man. *Gastroenterology* 1978;74:683–690.
 56. Bond JH, Levitt DG, Levitt MD. Quantification of countercurrent exchange during passive absorption from dog intestine—evidence for marked species differences in the efficiency of exchange. *J Clin Invest* 1977;59:308–318.
 57. Winne D. The vasculature of the jejunal villus. In: Kramer M, Lauterbach F, eds. *Intestinal permeation*. Amsterdam: Excerpta Medica, 1977:48–55.
 58. Hallböck D-A, Jodal M, Mannisheff M, Lundgren O. Tissue osmo-

- lality in intestinal villi of four mammals in vivo and in vitro. *Acta Physiol Scand* 1991;143:271-277.
59. Jodal M, Fihn B-M, Sjöqvist A. Transport of extracellular probes from lumen into plasma in the small intestine of the rat in vivo—effects of glucose. *Ger J Gastroenterol* 1993;31:571.
60. Hall EJ, Batt RM. Differential sugar absorption for the assessment of canine intestinal permeability: the cellobiose/mannitol test in gluten sensitive enteropathy of Irish setters. *Res Vet Sci* 1991;51:83-87.
61. Jodal M, Hallbäck D-A, Lundgren O. Tissue osmolality in intestinal villi during luminal perfusion with isotonic electrolyte solutions. *Acta Physiol Scand* 1978;102:94-107.
62. Krugliak P, Hollander D, Schlaepfer CC, Nguyen H, Ma TY. Mechanisms and sites of mannitol permeability of small and large intestine in the rat. *Dig Dis Sci* 1994;39:796-801.
63. Jodal M, Fihn B-M, Sjöqvist A. Effect of glucose on passive trans-

port of extracellular probes across the rat small intestinal epithelium in vivo (abstr). *Gastroenterology* 1994;106:A241.

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Address requests for reprints to: Jack A. Groot, Institute of Neurobiology, University of Amsterdam, Kruislaan 320, 1098 SM Amsterdam, The Netherlands. Fax: (31) 20-665-9125.

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