Disaccharide Feedings Enhance Rat Jejunal Macromolecular Absorption

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Summary

Disaccharide feedings to post-weaning rats alters their jejunal barrier to macromolecular absorption. Penetration of horseradish peroxidase (HRP) across the jejunum was enhanced after short-term high concentration gavage ($30 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) of lactose that produces weight loss, osmotic diarrhea, and jejunal mucosal damage. HRP absorption was also increased by longer term feedings of lower levels of disaccharide that did not produce body weight alterations, diarrhea, or cell damage. Rats without diarrhea and gavaged with $7.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ of either lactose or maltose showed an increase in lumen to blood HRP absorption after 21 days of feedings. Also, lactose or maltose in the solid food diet at $30 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for 21 days did not lead to diarrhea but produced an increase in jejunal lumen to blood HRP absorption. Rats having no diarrhea and receiving $15 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ of lactose or maltose in the drinking water for 21 days, showed an increase in jejunal HRP absorption. When rats were fed either lactose or maltose for 21 days glucose absorption became totally dependent upon the sugar concentration in the perfusion medium. The kinetics of this glucose absorption are compatible with a decrease in the "unstirred" layer. Disaccharide-induced HRP absorption may be mediated by transport from endocytotic vesicles across the jejunal epithelium.

Abbreviations

HRP, horseradish peroxidase
MVBs, multivesicular bodies
PEG, polyethylene glycol

Diarrheal disease in infancy is frequently associated with disaccharide intolerance (26, 27). There may also be an increased absorption of dietary protein (17) and, in some cases, development of milk and/or soy protein hypersensitivity (1, 11, 13, 14, 19, 20, 23). Some studies suggest that milk protein intolerance can be a secondary result of lactose intolerance (7, 14, 24, 29). Alternatively, other investigators reported data consistent with the view that disaccharide intolerance can be a secondary effect of milk protein hypersensitivity (20, 23, 30, 31). Disaccharide malabsorption produces luminal hyperosmolar gradients and intestinal fluid secretion (25, 26). In experimental animals, jejunal hyperosmolar gradients induce sodium and water secretion (10, 45) and increases lumen to blood macromolecular permeability (10).

The present study was designed to determine the effect of disaccharide feedings on the lumen to blood permeability of the upper small intestine to macromolecules. Rat jejunal absorption of HRP, a 40,000 molecular weight protein tracer, was studied after disaccharide feedings at concentrations that did or did not produce diarrhea. Our observations strongly suggest that disaccharide feedings increase jejunal permeability to HRP. This is apparent after short term feeding periods of a high lactose concentration, which produce diarrhea, and after more extensive

periods of feeding at lower disaccharide concentrations, which do not induce symptoms of intolerance. After the high lactose concentration feedings, there was evidence of morphologic damage to the epithelium of the jejunal mucosa. The disaccharide-induced increase in HRP absorption may be mediated by transport from endocytotic vesicles across the epithelium (10, 12, 43).

MATERIALS AND METHODS

Animals and diet. Postweaning male rats (CrI: (WI) BR, Charles River Breeding Labs, Wilmington, MA) weighing 100–150 g were used. At this stage of development the rats have low lactase levels (36). All rats were fed a powdered Purina Rodent Chow (#5001, Ralston Purina Co., St. Louis, MO) and water *ad libitum* throughout the study. The Purina chow diet contained 50% unrefined complex carbohydrate, 23.0% protein, 6.5% fats, and 6.8% minerals. The balance corresponded to fiber, vitamins, and moisture. In addition to this diet, disaccharides were experimentally administered to the rats.

Disaccharide administration by gavage with production of diarrhea. In one set of experiments lactose, sucrose or maltose was administered to the rats by gavage at $30 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for 1 and 7 days. This level of disaccharides was given as a 60% w/v slurry at 5 ml/100 g body weight, carefully resuspended before administration. Controls received 0.9% isotonic saline. The lactose-gavaged animals developed a severe osmotic diarrhea.

Disaccharide administration by gavage in the absence of diarrhea. A second set of rats received lactose or maltose by gavage at $7.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for 7 and 21 days. These disaccharides were delivered as a 15% w/v solution at 5 ml/100 g body weight. Controls received 0.9% isotonic saline. There was no evidence of diarrhea in animals fed this level of disaccharide (Table 1).

Disaccharide administration in solid food in the absence of diarrhea. Another group of rats received lactose or maltose at $30 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ as a 16.7% purified disaccharide supplement to the solid food diet.

This level was achieved by adding 20 g of disaccharide to 100 g of powdered commercial diet. Because the original carbohydrate concentration of the commercial diet was 50%, the addition of 20 g of disaccharide diluted the concentration of all nutrients, so that the final complex carbohydrate concentration was 41.7% and the disaccharide 16.7%. Fats and protein were also diluted to 5.4% and 19.2%, respectively. In this set of experiments controls were not precisely comparable to disaccharide fed rats because they received a standard Purina diet.

Disaccharide administration in the drinking water in the absence of diarrhea. Another group of rats received $15 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ of lactose or maltose *ad libitum* in their drinking water for 21 days. This disaccharide load was given as a 5% w/v solution in approximately 30 ml of fluid intake/day/rat. Controls received tap water. The rats drinking lactose or maltose also did not develop diarrhea (Table 1). Because maltose induced polydypsia (78 ml water

Table 1. Effect of lactose feedings on stool output, water, and food intake¹

	(Stool g/day)	Water (ml/day)	Food (g/day)
Gavage			
Lactose 15%	4.90 ± .36	27.31 ± 2.16	14.69 ± 1.06
Lactose 5%	5.18 ± .38	26.00 ± 1.82	15.44 ± 0.87
Saline	5.14 ± .35	28.25 ± 1.82	15.92 ± 0.81
Drinking			
Lactose 5%	4.37 ± .38	28.67 ± 2.30	14.33 ± 1.31
Water	5.27 ± .37	29.78 ± 2.02	15.86 ± 0.89
Diet			
Lactose 16.7%	4.58 ± .40	28.39 ± 1.97	14.28 ± 0.79

¹ Table 1 shows the absence of an effect of 5–16.7% lactose feedings on carbohydrate intolerance. Carbohydrate intolerance was defined by an increase in stool output and water intake. Rats ($n = 12$) in individual metabolic cages were cycled through six, 3-day lactose treatments in random order; all 12 rats experienced each treatment. Stool output, water and food intake were monitored daily. Using analysis of variance and Tukey's test for paired comparisons, no significant differences were found among any of the groups. $n = 36$ observations/treatment (means ± S.E.).

intake/day versus lactose, 31 ml/day and water controls, 29 ml/day; $P < 0.001$), we carried out a pair-drinking study. In this experiment the amount of fluid intake allowed to the rats offered maltose was matched with the amount consumed by the rats that drank the lactose solution.

Body weight stool output, water, and food intake. The only group of rats that failed to gain weight was the 7-day 30 g/kg lactose-gavaged animals. After 7 days these lactose-gavaged rats weighed 124 ± 5.2 g versus controls 163 ± 7.8 g; $P < 0.05$). All other disaccharide-fed rats gained weight as well as controls. When lactose was administered at $30 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$, by gavage, the rats had a severe watery diarrhea. Losses could not be quantitated in these stools because they were mixed with urine outputs. However, rats fed the lower disaccharide concentrations (5–16.7%) did not show any obvious evidence of diarrhea. In order to determine whether there were more subtle clinical signs of disaccharide intolerance, induced by the lower dose disaccharides, we carried out a separate study to monitor the effects of 5–16.7% lactose on stool output, water, and food intake (Table 1). Lactose was given by gavage, drinking water, or solid food. Twelve rats in individual metabolic cages were cycled through six, 3-day treatments in random order; all 12 rats experienced each treatment. Stool output, water, and food intake were monitored daily and were not affected by any of the disaccharide treatments. Statistical analysis was by analysis of variance with Tukey's test for paired comparisons.

Intestinal perfusion procedures. After the experimental feeding periods the rats were anesthetized with urethane (1.2 g/kg), by intraperitoneal injection, and a 30–40 cm jejunal segment was perfused *in vivo*, as described in detail elsewhere (10, 28, 49). In brief, the perfusion was carried out for 60 min at a rate of 0.19–0.22 ml/min with an isotonic solution (280 mOsm/kg) consisting of 144 mEq/liter of NaCl, 3 mM glucose, 600 mg/100 ml PEG (molecular weight, 3000–3700), 0.5 g/100 ml (125 μM /liter) HRP (Type II, Sigma Chem. Co., St. Louis, MO), all adjusted to a pH of 6.9.

A 60-min perfusion time was chosen because we previously demonstrated that experimental differences in HRP uptake into the circulation became apparent in perfusions by this time (10, 12). Our previous data demonstrated that it takes 30 min (10, 12) until HRP levels in the jejunal lumen reach a steady state in these perfusions.

HRP absorption - biochemical analysis. After 60 min of perfusion, serum HRP was determined by enzymatic methods described in detail elsewhere (12, 40). Aortic blood was allowed to flow into the barrel of a 3-ml heparinized syringe, with a 22-gauge heparinized needle, without a plunger to avoid hemolysis. Serum was separated and purified by two 10-min centrifugations at $500 \times g$.

Serum HRP activity was determined as described by Steinman and Cohn (40). This method monitors the rate of change in absorbance at 460 nm of *O*-dianisidine as it is oxidized. As noted previously (12, 40), activity is linear for up to 3 min. The sensitivity of the assay in our hands was 0.010 units. Exogenous HRP, added to fresh unexposed rat serum, gave a linear response that was proportional to the concentration of added HRP. HRP activity was expressed as $\mu\text{moles of H}_2\text{O}_2$ decomposed/min/ml serum/cm of jejunum perfused $\pm$ S.E. and corrected for a constant perfusion rate of 0.2 ml/min. Comparisons among animals on a specific disaccharide experimental protocol were expressed as % increase over controls for that treatment. As we previously reported (12), the peroxidase activity of serum prepared as above from rats not treated with HRP is very low and significantly less than the peroxidase activity of HRP-treated controls; thus, we are comparing serum levels of HRP reflecting jejunal perfusion with the tracer and not endogenous blood peroxidases. The measurements of intestinal length perfused were done with a constant applied tension of 3 g. We documented previously the direct proportionality of intestinal length and mucosal DNA under these conditions (12).

Glucose transport kinetics. The kinetics of intestinal transport of glucose were studied in rats fed a diet containing 16.7% lactose or maltose, as described above. The rats were perfused as detailed previously (28, 49) and the intestinal absorption of glucose determined *in vivo* in 20–30 cm jejunal segments perfused with two modified Krebs-Ringer buffers in randomized order. Glucose was perfused at concentrations of 4, 20, 40, 80, and 160 mM and at a rate of 0.19–0.22 ml/min. Ten perfusate samples from each solution during the steady state period were collected. Isotonicity was maintained by the adjustment of NaCl. Trace amounts of [¹⁴C]-U-D-glucose (10,000–20,000 dpm/ml) (New England Nuclear, Boston, MA) was added for estimation of glucose absorption by isotope dilution. PEG was included in the perfusion as a marker to calculate net water fluxes for glucose transport data. The methods are described in greater detail elsewhere (49, 50).

Cytochemistry, light and electron microscopy. HRP penetration across the jejunal epithelium was monitored by light and electron microscopic cytochemical procedures described elsewhere in detail (10, 12). Segments (2–3 cm) studied for cytochemical HRP penetration were at least 15 cm from any of the surgical sites required for cannulation (38). Fixation was with a cold solution of 2.5% glutaraldehyde and 1% sucrose in 0.1 M cacodylate buffer pH 7.3 for 60 min. Tissue was then rinsed in cold buffer containing 7% sucrose, frozen on the head of a freezing microtome, and incubated in the Graham and Karnovsky medium (10, 12, 16) for 60 min at room temperature. Enzyme cytochemical incubations were terminated by rinsing with cold 7.5% sucrose. The tissue was then postfixed in osmium tetroxide and prepared for light and electron microscopy by our routine methods (10, 12).

In rats not exposed to HRP and incubated under the conditions described above, there was no localization of reaction product at sites that ordinarily show the tracer. Reaction product in these HRP-free preparations is restricted to erythrocytes that contain hemoglobin, which is peroxidatic, and granulocytes, which contain endogenous myeloperoxidase.

Epon thick sections (1–2 μm) were studied unstained by phase contrast light microscopy for the localization and penetration of HRP. For each of the rats studied, 12–20 villi were examined by light microscopy. Electron microscopic analysis focused on the route of HRP penetration and on any alteration in the integrity of cellular organelles; therefore, in the electron microscope study we focused on regions with clear penetration of HRP into the lamina propria of jejunal villi.

Thin silver-grey sections were studied by electron microscopy on a JEOL JEM-100 electron microscope, unstained or lightly stained with lead. Unstained sections were used because the stain may occasionally be confused with light reaction product for HRP. Electron micrographs were taken at initial magnifications of $\times 4000$ –20,000.

RESULTS

HRP absorption in the presence of diarrhea. The rats with diarrhea, which were gavaged with $30 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ lactose, showed an increased lumen to blood penetration of HRP across the jejunal epithelium (Table 2) as compared to saline-gavaged controls. This increased HRP absorption was apparent after 1 day and 7 days of lactose by gavage. Rats gavaged with $30 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ sucrose for 1 day also showed an increased jejunal absorption of HRP; however, they did not develop diarrhea and serum HRP levels were lower than in the lactose gavaged animals (Table 2). In rats fed the same concentration of maltose by gavage for 7 days there was no increase in jejunal HRP absorption.

HRP absorption in the absence of diarrhea. A. Gavage studies. Rats without diarrhea, which were gavaged with $7.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ of either lactose or maltose showed an increase in lumen to blood jejunal HRP absorption after 21 days of feedings (Table 2). HRP absorption was higher in the lactose-fed rats than those administered maltose. After a 7-day feeding period at $7.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$, lactose- and maltose-gavaged animals showed HRP absorption levels similar to those in saline-gavaged controls; there was no evidence of diarrhea.

HRP absorption in the absence of diarrhea. B. Solid diet studies. Lactose or maltose feedings in the solid food diet at $30 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for 21 days also did not lead to diarrhea, but produced an increase in jejunal lumen to blood HRP absorption as compared to controls fed no additional disaccharide (Table 2). This level of disaccharide feedings did not increase jejunal HRP absorption as compared to controls after 7 days of feeding.

C. Drinking water studies. Rats with no diarrhea that received $15 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ of either lactose or maltose in the drinking water for 21 days, showed an increase in jejunal HRP absorption above that seen in rats drinking plain tap water (Table 2). When maltose was administered *ad libitum*, the rats became polydipsic and showed jejunal HRP absorption levels even greater than in the lactose group. Pair-drinking (Table 2) eliminated the differences between the lactose- and maltose-fed rats. Both groups of animals showed serum HRP levels, after perfusion, that were significantly greater than in controls drinking water alone.

Glucose transport kinetics. Changes in the general permeability and transport properties of the jejunum were evaluated by studying the effect of longer term (21 day) disaccharide diets on glucose

transport kinetics (Fig. 1). Both maltose and lactose in the solid diet significantly altered glucose transport kinetics. A graphic representation of the data, which plotted the ratio of glucose concentration to the transport rate (S/V) against the glucose concentration (S), was selected because this transformation permits a rapid discrimination of mediated (active) and non-mediated (diffusional) transport (34). The control rats exhibited a correlation between concentration (S) and S/V , consistent with active transport being the primary mode of absorption (Fig. 1). In contrast, the rats fed either lactose or maltose appeared as if their

Fig. 1. Plot of glucose transport kinetics (S/V vs S) for small intestinal glucose transport of rats fed a Purina chow diet containing an additional 16.7% lactose or maltose for 3 wk, or a control Purina diet with no additional carbohydrate. For the controls ($\bullet$), the linear regression of the data has a significant correlation ($r = 0.772$, $P < 0.001$). K_t was calculated to be 54.4 mM, V_{max} , 245.9 nmole/min/cm. In contrast, neither the lactose-fed ($\times$) or maltose-fed ($\circ$) rats show a significant correlation ($P > 0.1$) between S and S/V , and no K_t or V_{max} can be calculated. Glucose transport in lactose- and maltose-fed rats appears predominantly diffusional and non-carrier mediated. The plot of S/V vs S was chosen because it can reveal a large diffusion component of transport; diffusion tends to produce a horizontal line (34). There were 16 rats/group, a total of 48 rats. Each point represents the means $\pm$ S.E. of 20–40 determinations in the separate samples obtained from the perfusates.

Table 2. Serum HRP levels after jejunal perfusions of rats that were maintained experimentally on disaccharides for varying amounts of time as indicated

Disaccharide used	Administration mode	Disaccharide level	Feeding duration (days)	Diarrhea (Yes/No)	Serum HRP ¹ (% Control)	P
Lactose	Gavage	60% (30 g/kg)	1	Yes	224 $\pm$ 16	<0.01
Sucrose	Gavage	60% (30 g/kg)	1	No	179 $\pm$ 11	<0.05
Lactose	Gavage	60% (30 g/kg)	7	Yes	152 $\pm$ 25	<0.05
Maltose	Gavage	60% (30 g/kg)	7	No	83 $\pm$ 30	NS
Lactose	Gavage	15% (7.5 g/kg)	7	No	106 $\pm$ 22	NS
Maltose	Gavage	15% (7.5 g/kg)	7	No	99 $\pm$ 11	NS
Lactose	Gavage	15% (7.5 g/kg)	21	No	276 $\pm$ 14	<0.01
Maltose	Gavage	15% (7.5 g/kg)	21	No	207 $\pm$ 19	<0.05
Lactose	Diet	16.7% (30 g/kg)	7	No	69 $\pm$ 17	NS
Maltose	Diet	16.7% (30 g/kg)	7	No	57 $\pm$ 36	NS
Lactose	Diet	16.7% (30 g/kg)	21	No	239 $\pm$ 9	<0.001
Maltose	Diet	16.7% (30 g/kg)	21	No	183 $\pm$ 16	<0.05
Lactose	Drinking	5% (15 g/kg)	21	No	201 $\pm$ 21	<0.05
Maltose ²	Drinking	5% (15 g/kg)	21	No	246 $\pm$ 25	<0.01
Maltose ³	Drinking	5% (15 g/kg)	21	No	196 $\pm$ 19	<0.05

¹ Serum horseradish peroxidase (HRP) levels are given as % of values of their respective controls $\pm$ S.E. within each experimental group. Gavaged controls received isotonic saline, diet controls had no additional disaccharide, and drinking controls received pure tap water, $n = 6$ –12 rats/group.

² *Ad libitum* drinking.

³ Pair drinking.

Fig. 2a-c. Phase contrast light photomicrographs of jejunal villi from experimental rats gavaged with $30 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ (60%) lactose (2a and 2b) for 7 days or a control rat gavaged with isotonic 0.9% saline (2c) for 7 days. After the experimental feeding periods the jejunum of each of the rats was perfused *in vivo* with an isotonic solution containing 0.5 g% horseradish peroxidase (HRP). In (2a), from a lactose gavaged animal, reaction product for HRP is seen on the brush border (B), in the intercellular spaces between adjacent epithelial cells (arrows) and in the lamina propria (LP) of the villus. Note that the villus is histologically intact. Goblet cell is at G, intestinal lumen is at L. In (2b), also from a lactose-gavaged rat, the villus shows marked cytochemically demonstrable penetration of HRP (arrows) into the LP. There also appears to be some evidence of focal flattening of the absorptive epithelium (arrowhead). Brush border is at B, jejunal lumen at L. In (2c), from a saline control, note that reaction product for HRP is confined to the brush border (B) of the villus and there is no penetration of the tracer into the LP. Intestinal lumen is at L. Goblet cell mucus at G. 2a, $\times 640$; 2b, $\times 640$; and 2c, $\times 640$.

glucose absorption capacity was totally dependent upon the sugar concentration in the medium, *i.e.*, diffusion. Under these conditions, the kinetic constants for the disaccharide-fed rats could not be determined. For the control rats, the graphically calculated K_t was 54 mM and the $V_{\max}$, 246 nmoles/min $\times$ cm. The affinity constant compared well with the value obtained in other studies using the same *in vivo* technique (28, 50).

Light and electron microscope cytochemistry. HRP penetration across the jejunal epithelium and into the lamina propria was most dramatic by light and electron microscope cytochemistry after $30 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ lactose, administered by gavage for 1 or 7 days (Figs. 2 and 3). In these preparations, reaction product for HRP was seen on the microvillar brush border between epithelial cells and in the lamina propria (Figs. 2 and 3). By contrast, HRP was confined to the brush border of epithelial cells in saline-gavaged animals (Fig. 2c). In these 60% lactose-gavage rats, there was evidence of focal damage to the jejunal epithelium (Figs. 2b and 4), although the majority of villi remained entirely structurally intact. Some epithelial cells from rats gavaged with 60% lactose for 1 or 7 days contained an increased number of lysosomes with autophaged material (Fig. 4).

HRP absorption was also cytochemically demonstrable at focal sites after gavage lactose or maltose feedings of 7.5 g/kg for 21 days, solid diet feedings of 30 g/kg lactose or maltose for 21 days (Figs. 5 and 6) and drinking water disaccharide administration at 15 g/kg for 21 days. On the other hand, in saline-gavaged, dietary or drinking water controls, HRP was almost entirely confined to the microvillar surface and a few intracellular vesicles and multivesicular bodies. The tracer was only very rarely demonstrable in the intercellular space.

Where HRP penetration across the jejunal epithelium was demonstrable, the tracer was seen on the microvillar brush border, in endocytotic vesicles in the terminal web zone and in the lateral intercellular space between adjacent epithelial cells (Figs. 3, 5, 6). Very occasionally, an HRP-filled endocytotic vesicle was seen in

close proximity to the lateral plasma membrane of the epithelial cell (Figs. 3 and 5). Many of the jejunal epithelial cells with demonstrable HRP penetration into the intercellular space also contained numerous HRP-filled MVBs (Fig. 3). But in some preparations there were numerous HRP-filled MVBs and endocytotic vesicles in the terminal web zone and little or no tracer in the intercellular spaces. In other cells there was strong reaction product for HRP in the intercellular spaces (Figs. 5 and 6) with only an occasional endocytotic vesicle in the terminal web zone of the epithelium. Microfold cells were only very rarely encountered in these jejunal preparations.

There was no evidence of focal histologic or ultrastructural cellular damage with disaccharide feedings at $7.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ gavage, $30 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ in the diet or $15 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ in the drinking water. Although there were occasional sites of villus tip cellular exfoliation, the numbers of these cells were indistinguishable from those in saline- or water-fed controls. Under all of these conditions, there was no loss of villus integrity and organelles of the epithelial cells were unremarkable in appearance (Figs. 5 and 6).

DISCUSSION

Disaccharide feedings and HRP absorption. The key point of our observations is that feedings of disaccharides to postweaning rats alter the jejunal barrier to macromolecular absorption. HRP penetration across the jejunum was enhanced after gavage short term, high concentration ($30 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) lactose feedings that produced weight loss, osmotic diarrhea, and jejunal mucosal damage. HRP absorption was also increased by longer term lower levels of disaccharide feedings that produced no body weight alterations, no diarrhea and no cell damage. These effects of disaccharide feedings of HRP permeability are apparently not due to their mode of administration: they were seen after gavage, dietary disaccharide, and disaccharide in the drinking water. Further-

Fig. 3. Electron micrograph of the epithelium from a jejunal preparation gavaged with $30 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ (60%) lactose for 7 days and then perfused *in vivo* with horseradish peroxidase (HRP). Reaction product for HRP is demonstrable on the microvillar surface (V), in numerous pinocytotic vesicles (P) and multivesicular bodies (MV) in the terminal web (TW) region. Intense reaction product for HRP is also seen in the intercellular spaces (arrows) between the jejunal epithelial cells. Mitochondria are at M and the jejunal lumen at L. In Figure 3 inset A, a portion of the jejunal surface is shown at a higher magnification. Note the reaction product for HRP in a pinocytotic tubule and vesicle (P) seen in very close association with the lateral plasma membrane (PM) of the epithelium. Reaction product for the tracer is also seen in the immediately adjacent intercellular space (arrow) as well as on the microvillar brush border (V). There is no reaction product for HRP in the tight junctional zone (T). Images such as these suggest a role for "bulk" endocytotic transport of HRP in these preparations. Figure 3 $\times 11,000$ and 3A, inset $\times 38,000$.

more, the increased HRP absorption after gavage is not due merely to alterations induced by the mechanical force of feedings because the effect is not seen in saline-fed controls or rats fed the lower concentrations of disaccharide for shorter periods of time. The fact that animals fed the lower doses of disaccharide gained weight and had food and water intake comparable to controls indicates that our observations are not due to some alteration in the nutritional status or to dehydration of the animals.

Clearly, the rate at which the disaccharides are administered does play some role in the time it takes to alter jejunal permeability to HRP; thus, $30 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ of lactose, delivered as a single bolus by gavage, most rapidly alters the jejunal macromolecular barrier. On the other hand $30 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ of disaccharide, administered by means of a solid food diet over a 24-h period, is less

rapidly effective and takes up to 21 days to produce jejunal macromolecular permeability changes. These observations are expected because the single bolus of an osmotic load given by gavage route most severely stresses intestinal integrity and morphology (45).

The enhanced jejunal permeability to macromolecules induced by these disaccharide feedings may be the result of one of several processes. The luminal hypertonic gradient that can be generated by hyperosmolar feedings such as those used in the present study may have a direct damaging effect on the mucosal barrier to macromolecules: direct jejunal perfusion of hypertonic mannitol permits increased jejunal penetration of HRP (10, 43), as the gut lumen moves towards isotonicity by secreting water. Osmotic equilibration also has been reported when the rat is gavaged with

Fig. 4. Portion of jejunal epithelial cell from a rat gavaged with $30 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ (60%) lactose and perfused as in Figure 3. A large lysosome (LY) containing membranous fragments including a partially intact mitochondrion (M) is seen. Two multivesicular bodies are at MV, and reaction product for HRP is seen in the intercellular space (arrows). This type of image suggests that the jejunal epithelium may have undergone some type of membrane-related damage, stimulating autophagic organelle turnover (21, 22). A similar type of damage is seen in hypertonically perfused (10) or gavaged preparations (45). $\times 30,000$.

Fig. 5. Electron micrograph of the jejunal epithelium from a rat fed lactose in the solid food diet at $30 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}$ (16.7%) for 21 days, followed by *in vivo* intestinal perfusion with HRP. HRP is demonstrable on the microvillar surface (V), in pinocytotic vesicles (P) that occasionally appear as if forming at the base of the microvilli, and in the intercellular space (arrows), between epithelial cells. The jejunal lumen is at L, mitochondria are at M. In the inset (5A), from an identically treated jejunal preparation, HRP is seen on the microvillar surface (V), in a pinocytotic vesicle closely associated with the lateral intercellular space (arrow) that is filled with HRP. Note the absence of reaction product for HRP in the most apical tight junctional (T) zone between the epithelial cells. Figure 5, $\times 23,500$ and 5A, $\times 40,000$.

Fig. 6. Jejunal epithelium from a rat that was fed maltose at $30 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ (16.7%) for 21 days and then underwent *in vivo* intestinal perfusion with an isotonic solution containing horseradish peroxidase (HRP). Reaction product for HRP is seen on the microvillar surface (V) and in the intercellular space (arrows) between the epithelial cells. Mitochondrion at M, mucus droplets (MU) in a goblet cell. $\times 11,000$.

hypertonic mannitol (45). Other macromolecular barriers, such as the blood-brain barrier (6) and hepatocyte junctions (15) are also sensitive to hypertonic disaccharides. Thus, disaccharides delivered in the present study may have generated chronic transient luminal hyperosmolar gradients that altered jejunal epithelial permeability. Because the rats we used are lactase deficient, this is most likely to be the case with the lactose administration studies and it may also occur when, theoretically more absorbable disaccharides, such as maltose, are delivered in quantities that exceed their ability to be hydrolyzed and absorbed.

The alteration in macromolecular permeability produced by these disaccharide feedings might also be related to changes induced in the properties of the jejunal surface. The prevalence of diffusional, non-carrier mediated, glucose transport produced by 3 wk of solid food disaccharides is compatible with an obliteration of active transport and decreased resistance in the physiologic "unstirred" layer (51, 52). This might permit easier access of macromolecules to the jejunal surface and lead to an increased jejunal permeability. We believe that the disaccharide-feeding induced diffusional glucose transport is not merely due to some increase in mucosal permeability to the water flux marker, PEG, used in this analysis. If PEG loss were an important factor there would be a reduction in the calculated glucose transport and the plot of S/V versus V would more nearly resemble controls. Any PEG loss that may have occurred was insufficient to produce such an effect on glucose transport. It may be relevant that there is an increase, which is induced by lactose feedings, in mineral absorption that is believed to be mediated by a nonselective increase in passive ion diffusion (8, 32).

Alternatively, the longer term alteration in macromolecular permeability induced by disaccharide feedings might be the result of some bacterially mediated process. The population of bacterial

flora in the intestinal lumen may be altered by the excess free carbohydrates (9, 37). These flora may proliferate and generate injurious products (4, 12, 43, 44) that could alter the permeability of the jejunal epithelium.

Route of HRP absorption. The precise locus of the alteration in the macromolecular barrier induced by the disaccharide feedings is not entirely clear. Increased lumen to blood HRP penetration may be the result of diffusion of the tracer across dying cells (18), to a "washing" off of the secretory antibody or mucus barrier (47, 48), to increased rates of bulk transport in endocytotic vesicles (10, 12, 39, 44) or to an altered tight junctional barrier (10, 12, 38, 44).

When gavaged with a high level (30 g/kg) of lactose, damage to the jejunal epithelium could in part account for the rapidly altered macromolecular permeability. With the lower dose of disaccharide feedings, the numbers of dying cells were relatively small and indistinguishable from controls. Because the cytochemical observations do not permit a quantitative assessment of HRP penetration, we cannot rule out the possibility of some increased diffusion rate of HRP under these conditions. The combination of unremarkable numbers of diffusely stained cells and the lack of morphologic evidence of cell damage, support the view that diffusion does not appreciably account for the increased macromolecular permeability.

In contrast to our previous direct hypertonic perfusion studies (10), we did not find evidence of HRP demonstrable within the tight junctional zone of the jejunal epithelium in the present study.

We think that the most probable route involved in the disaccharide-induced jejunal HRP absorption is bulk flow in endocytotic vesicles. HRP-labeled vesicles were seen in epithelial cells with demonstrable penetration of the tracer into the lamina propria. The labeled vesicles appeared to be forming at the base of microvilli and were seen in association with the lateral intercellular

space, in images suggesting a shuttling from the microvillar surface to the lateral intercellular space, where their content could be released by exocytosis (39, 43).

Although many of the epithelial cells with HRP penetration into the intercellular space contained tracer-labeled multivesicular bodies (MVBs) (21, 22), some preparations with extensive endocytosis and no tracer penetration into the intercellular space also contained many MVBs. An association between luminal hyperosmality, endocytosis and hormone-mediated water absorption has been reported in toad urinary bladder epithelium (33, 46). The exact relationships among rates of endocytosis, MVB formation and HRP absorption in the intestinal epithelium clearly require further study.

It should be noted that in this study HRP penetration occurred across regular absorptive epithelial cells. Although abundant in the ileum (35), apparent microfold cells were only very rarely encountered in the proximal jejunum of the rats which we studied.

Clinical implications. Our observations hint that feedings of poorly absorbed carbohydrates, such as lactose, can increase jejunal absorption of intact, potentially antigenic, food protein. Diarrheal disease, carbohydrate intolerance, and food protein intolerance are frequently associated (7, 14, 19, 20, 23, 30) although some questions remain concerning the initiating event. Our data are consistent with the possibility that disaccharide malabsorption might eventually increase intact protein absorption and lead to food protein intolerance in a genetically susceptible host (11, 13, 14, 17, 47, 48). This does not preclude the possibility that food protein intolerance can trigger carbohydrate intolerance (20, 23, 30, 31) or that infection (5) can also increase intestinal absorption of intact protein. Furthermore, ontogenetically lactase-deficient individuals, who do not experience symptoms of intolerance (3, 41), may be at an increased risk for the absorption of protein antigens when fed milk over long periods of time. This hypothesis remains to be more directly tested.

Clearly, the intestinal barriers of rats and humans are not necessarily analogous and much caution is warranted in extrapolation of rat experimental data to humans. A 40-kg child who drinks four glasses of milk consumes 1.5 g/kg of lactose. Bearing in mind that rats are generally ten times (2) more resistant to the effects of damaging agents than humans, finding noxious effects of lactose feedings at a 5 g/kg level in rats, may be relevant to human nutrition.

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