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Research Article

To identify the site of stimulation of sucrase by a sucrose diet, changes in sucrase-specific activity of jejunal mucosa were studied after introduction of sucrose diet to carbohydrate-deprived rats. Results were correlated with simultaneous changes in villus gradients of sucrase-specific activity. Simultaneous with the introduction of sucrose diet, [^{3}H]thymidine (100 μCi) was administered intravenously, and rates of cell migration measured during adaptation to the new diet. After a 72-h fast, rats fed sucrose diet for 6, 12, or 18 h showed no change in sucrase-specific activity in either whole mucosa or villus gradients. However, within 18-24 h after starting a sucrose diet, there was a marked rise in whole mucosal sucrase-specific activity above fasting values (99 ± 14 vs. 38 ± 4 μM glucose/min per g protein, $P < 0.001$) in association with the development of a region of increased activity at the lower villus (154 ± 22 vs. 60 ± 9 μM glucose/min per g protein, $P < 0.02$, but with no change in villus tip activity (56 ± 5 vs. 46 ± 8 μM glucose/min per g protein). Similar changes were seen in animals fed 24 h of sucrose diet after a 72-h carbohydratefree diet. Fasted animals fed sucrose diet for 36 h had increased sucrase-specific activity at the villus tip (144 ± 11 μM glucose/min per g […]

Site of Substrate Stimulation of Jejunal Sucrase in the Rat

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ABSTRACT To identify the site of stimulation of sucrase by a sucrose diet, changes in sucrase-specific activity of jejunal mucosa were studied after introduction of sucrose diet to carbohydrate-deprived rats. Results were correlated with simultaneous changes in villus gradients of sucrase-specific activity. Simultaneous with the introduction of sucrose diet, [^{3}H]thymidine (100 μCi) was administered intravenously, and rates of cell migration measured during adaptation to the new diet. After a 72-h fast, rats fed sucrose diet for 6, 12, or 18 h showed no change in sucrase-specific activity in either whole mucosa or villus gradients. However, within 18–24 h after starting a sucrose diet, there was a marked rise in whole mucosal sucrase-specific activity above fasting values (99±14 vs. 38±4 μM glucose/min per g protein, $P < 0.001$) in association with the development of a region of increased activity at the lower villus (154±22 vs. 60±9 μM glucose/min per g protein, $P < 0.02$, but with no change in villus tip activity (56±5 vs. 46±8 μM glucose/min per g protein). Similar changes were seen in animals fed 24 h of sucrose diet after a 72-h carbohydratefree diet. Fasted animals fed sucrose diet for 36 h had increased sucrase-specific activity at the villus tip (144±11 μM glucose/min per g protein) as well as at the lower villus region, and this pattern persisted at 1 wk of sucrose diet. Maximal activity patterns for isomaltase and maltase paralleled those for sucrase, but the villus gradients for lactase were unaffected by sucrose diet. The region of maximal sucrase-specific activity always coincided with or followed the leading edge of radioactivity as determined by liquid scintillation counting. Therefore, sucrose-mediated changes in sucrase activity of the jejunal mucosa in the rat appear to be initiated at the level of the crypt epithelial cell and are expressed after a latent period of 18–24 h during which these cells mature and migrate toward the villus tip.

INTRODUCTION

Despite numerous studies indicating that dietary carbohydrate increases intestinal disaccharidase activity, the mechanism by which this occurs remains obscure (1–5). There is general agreement that jejunal sucrase activity falls during total fasting or carbohydrate deprivation (1, 2). Indeed, in the rat, after such treatment, sucrose feeding for 24 h leads to a twofold rise in jejunal sucrase activity, as well as a small but significant rise in maltase activity (1, 2). Conversely, maltose feeding stimulates a marked increase in maltase activity but a lesser rise in sucrase activity (2). With either diet, lactase activity remains unchanged in comparison to control animals (2). Similarly, human studies have shown a decrease in jejunal sucrase activity on removal of sucrose from the diet and an increase on its reintroduction (3). Because the time-course of the increase or decrease of sucrase activity correlates with the small intestinal epithelial cell turnover time in the human, it has been suggested that the site of control of this process might be the crypt epithelial cell (4). However, direct evidence for this hypothesis is lacking.

Accordingly, we have studied the site of initiation of activation of sucrase by determining the time-course and localization of sucrose-stimulated changes in sucrase-specific activity in the proximal jejunum of the rat. Localization of sucrase activation and correlation with epithelial cell migration have been achieved with a technique of sectioning the mucosa from villus tip to crypt base (6). The findings suggest that it is the immature crypt epithelial cell rather than the mature

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villus cell that responds to changes in dietary sucrose, and that sucrase activity levels of the differentiated enterocyte are determined before maturation.

METHODS

Diet and animals. Adult male albino rats (Charles River Breeding Laboratories, Wilmington, Mass.; Sprague-Dawley derived) weighing 125–175 g were either fasted or fed a carbohydrate-free diet for a 3-d period. These rats were then divided into groups that were either sacrificed immediately or fed high sucrose diets for varying time periods before sacrifice, as noted in Results. To encourage acceptance, the rats fed the carbohydrate-free diet were fasted for 1 d before a dietary change. The carbohydrate-free (CHO-free)[1] diet (0% carbohydrate, 18% protein, 34% fat, 44% alpha cellulose, 4% vitamin and mineral mixture) and the sucrose diet (68% sucrose, 18% protein, 8% fat, 6% vitamin and mineral mixture) were isocaloric and were obtained from Nutritional Biochemicals Company, Cleveland, Ohio. All rats were kept in wire-bottom cages and were offered water ad lib. throughout the study.

Preparation of tissue. Under light ether anesthesia, rats were exsanguinated by severing the aorta. The second 10 cm of jejunum (measured from the ligament of Treitz) was excised and immediately placed in 25 ml iced 0.9% NaCl solution (adjusted to pH 7.4 with $NaHCO_3$) and subsequently rinsed with 25 ml of the same solution. The mucosa was scraped with glass slides on an iced glass plate, homogenized in 50 vol of 5 mM EDTA, pH 7.4, and stored at −40°C for subsequent enzyme assays.

The third 10 cm segment of jejunum (from the ligament of Treitz) was excised simultaneously and rapidly prepared for cryostat sectioning by a modification of the method described by Nordström et al. (6). The tissue samples were spread (serosa down) on bolting silk (E.J. Ardon Company, Boston, Mass.) and then were applied to cryostat blocks with OCT mounting medium (Ames Co., Div. of Miles Lab., Inc., Elkhart, Ind.) at −20°C using minimal traction. Only flat, undamaged samples were used. 20-μm cryostat sections were placed serially, two sections per tube, into 0.5 ml iced 5 mM EDTA, pH 7.4. The tubes were sequentially numbered from villus tip to crypt base. The first tube contained three sections to provide an adequate sample for analysis. However, on occasion this tube was found to contain a negligible quantity of protein and was then discarded as the contents were only the frozen residue of the saline solution used initially to irrigate the mucosa. Specimens were manually homogenized and stored at −40°C for subsequent assay. By these techniques, gradients of enzyme activity could be reproducibly obtained. To allow comparisons among the diet groups, the gradients were normalized to percentage of villus height as suggested by Nordström et al. (6). As sucrase activity was seen only in the villus region, the portion of the villus containing sucrase activity was divided into 10 equal parts and labeled 0–100% of the villus height (6). The first 10% of this new villus gradient represented the villus base, whereas 100% represented the villus tip region. Fig. 1 is a gradient of sucrase-specific activity from villus tip to base from a group of adult rats on laboratory chow diet and illustrates the manner in which the data are presented. When the level of enzyme activity was correlated with morphologic location, blocks were partially cut removing a known number of sections. They were then defrosted, placed in 10% formalin, embedded in paraffin, processed using routine histologic techniques, and stained with hematoxylin-eosin. In other experiments, 10-μm cryostat sections were taken from various levels in the block and stained to confirm position in the villus crypt gradient as suggested by Nordström et al. (6).

Radioactive labeling. When cell migration was studied, simultaneous with the initiation of sucrose feeding, animals received 100 μCi [*methyl*-^{3}H]thymidine i.v. From the cryostat homogenates prepared as described above, aliquots of equal volume were combined in groups of four to provide adequate sample for counting. These were solubilized in Protosol (New England Nuclear, Boston, Mass.) to which a standard toluene-PPO-POPOP mixture (Liquifluor, New England Nuclear) was added. Samples were counted in a Packard liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.), corrected for differential quenching by the external standard method (efficiency, 28%), and the counts normalized to disintegrations per minute per segment. With this technique, the leading region of radioactivity had migrated 54±3% at 24 h (n = 14) and 96±4% at 36 h (n = 3) after injection. This was similar to the rates of cell migration as determined by others (7, 8). Background radioactivity averaged about 20% of activity in the leading region.

Enzyme assays. Sucrase (invertase, EC 3.2.1.26), lactase (EC 3.2.1.23) and maltase (EC 3.2.1.20) activities were determined by the ultramicro method of Messer and Dahlqvist (9), modified as described (10). Similar techniques were used for the assay of palatinase (EC 3.2.1.10) activity, which was considered to reflect isomaltase activity. Results were expressed as micromoles of glucose liberated per minute per gram of protein or as millimicromoles per cryostat homogenate. Protein was determined by the method of Lowry et al. (11) using crystalline bovine serum albumin as a standard. Experimental results were compared with the standard Student's t test and were expressed as the mean±SEM.

Substrates and materials. Enzyme substrates were obtained as described (10). [*methyl*-^{3}H]Thymidine (20 Ci/mol) was purchased from New England Nuclear.

RESULTS

Preliminary experiments were performed to identify the time-course of the effect of sucrose diet on sucrase activity in whole mucosal homogenates of jejunum at various times after the onset of sucrose feeding, as shown in Fig. 2. Animals fed sucrose for 6, 12, and 18 h after a 3-d fast showed no change in mean sucrase-specific activity when compared to fasted animals (Fig.

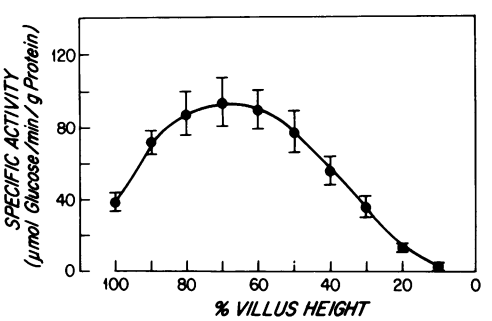

FIGURE 1 Sucrase-specific activity gradient along the villus in the jejunum of adult male rats on laboratory chow diet. Techniques are described in Methods. The data are mean±SE, n = 6.

[1] *Abbreviation used in this paper*: CHO-free, carbohydrate-free.

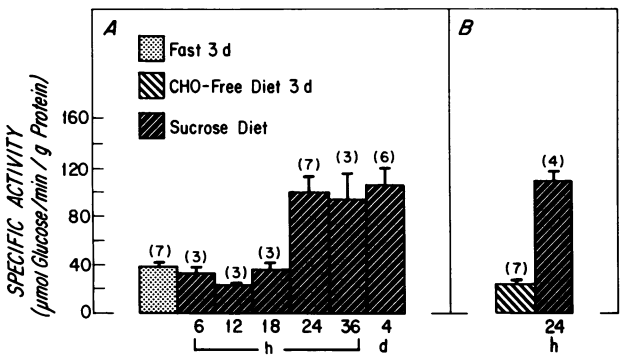

FIGURE 2 Effect of sucrose diet on mean sucrase-specific activity in whole mucosal homogenates of the jejunum. (A) animals were fasted for 3 d and then either sacrificed or fed sucrose diet for the time periods noted. (B) animals were fed CHO-free diet for 3 d and then either were sacrificed or fed sucrose diet for 24 h. The bracket represents the standard error of the mean. The numbers in parentheses indicate the number of separate experiments performed at each time point. Techniques are described in Methods.

2A). However, by 24 h there was a significant rise in mean sucrase-specific activity to 2.5 times the basal level ($P < 0.001$). This increased activity persisted in animals fed sucrose diet up to 7 d (not shown).

In rats fed the CHO-free diet for 3 d (Fig. 2B) mean sucrase-specific activity was not significantly different from that in fasted animals. When sucrose diet was fed for 24 h after the CHO-free diet there was a rise in mean jejunal sucrase-specific activity to four times the level of the animals on CHO-free diet alone ($P < 0.001$). The mean sucrase-specific activities in the groups fed sucrose diet for 24 h or more after either fast or CHO-free diet were not significantly different from one another.

The mean jejunal whole mucosal lactase-specific activity in the fasted group remained unchanged after 24 h of sucrose diet (34.1±5.9 vs. 37.8±4.7 μmol glucose/min per g protein). In contrast, the mean lactase-specific activity in animals fed CHO-free diet was significantly lower (8.2±1.8 μmol glucose/min per g protein, $P < 0.01$) than in the fasted group. Upon feeding sucrose for 24 h to animals previously fed the CHO-free diet, mean lactase-specific activity rose to 37.0±3.0 μmol glucose/min per g protein, $P < 0.001$, values no different from those found in fasted animals with or without sucrose feeding. Similar findings have been noted previously in this laboratory for rats on CHO-free diet (12).

The effect of sucrose feeding on the distribution of sucrase activity in villus gradients is shown in Fig. 3. Sucrase-specific activity is shown as mean±SEM at the villus height noted for all animals in a diet group. The data represent the progressive changes in the sucrase-specific activity gradients in animals fasted for 72 h and subsequently fed sucrose diet for the time periods shown. After a fast, sucrase-specific activity was at a basal level from villus tip to lower villus. At 12 and 18 h there was no significant change in the pattern of activity. However, at 24 h, there was a marked increase in sucrase-specific activity at the lower villus region (Table I). By 36 h of sucrose feeding, this increase in activity had progressed to the villus tip region; the pattern at 4 and 7 d (not shown) remained similar to that at 36 h. When [*methyl*-^{3}H]thymidine was injected at the onset of sucrose diet, the leading region of radioactivity had migrated 22±3% at 12 h, 43±3% at 18 h, 53±2% at 24 h, and 96±4% at 36 h. As indicated by the stippled bar (Fig. 3) it is apparent that the major region of increased sucrase activity coincided with the leading region of radioactivity.

To carry out a statistical analysis of the data, mean sucrase-specific activities at each normalized villus height (Fig. 3) were compared among the experimental groups with the Student's t test. The resulting values are shown in Table I. When rats were fed sucrose diet for 12 or 18 h, sucrase-specific activity was not statistically different from that in the fasted animals at any villus level. However, at 24 h a twofold increase in sucrase-specific activity had reached 60% of the villus height. This was coincident with the leading region of radioactivity that was at 53% of the villus height. By 36 h the significant elevation (twofold) of sucrase-specific activity in comparison to the fasted animals had reached the villus tip region and was coincident with the leading region of radioactivity (96%). At 4 and 7 d (latter not shown, $n = 4$) the pattern remained similar to that at 36 h of sucrose diet. When the 36 h, 4 and 7 d (not shown) sucrose diet groups were compared to the animals on 24 h of sucrose diet, the former groups had

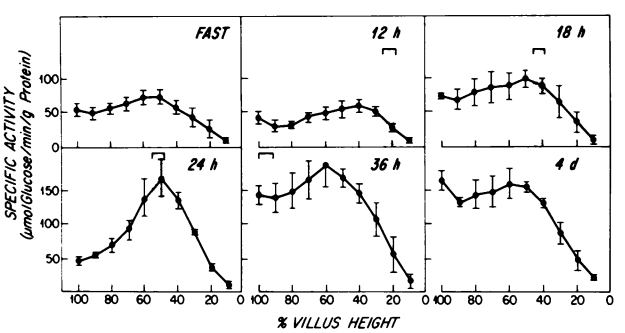

FIGURE 3 Effect of sucrose feeding after a 3-d fast on jejunal sucrase-specific activity gradients. Each panel shows the gradient of sucrase activity plotted against location on the villus. The data shown are mean±SE obtained as described in Methods: $n = 7$ (24 h), $n = 6$ (fast and 12 h), $n = 3$ (18, 36 h, 4 d). The stippled bar represents the leading region of radioactivity as described in Methods expressed as percentage of villus height: 12 h (22±3%), 18 h (43±3%), 24 h (53±2%), 36 h (96±4%).

TABLE I

*Statistical Analysis (P Values) of the Effect of Sucrose Feeding on Sucrase Activity along Villus**

Diet groups compared	Percentage of villus height									
	100	90	80	70	60	50	40	30	20	10
Fast (n = 6) to sucrose diet:										
24 h (n = 7)	NS	NS	NS	NS	<0.05	<0.02	<0.001	<0.01	NS	NS
36 h (n = 3)	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.05	NS	NS
4 d (n = 3)	<0.001	<0.001	<0.005	<0.01	<0.01	<0.005	<0.005	NS	NS	NS
Sucrose diet 24 h to:										
36 h	<0.001	<0.001	<0.01	<0.05	NS	NS	NS	NS	NS	NS
4 d	<0.001	<0.001	<0.01	NS	NS	NS	NS	NS	NS	NS
CHO-free diet (n = 3) to:										
Sucrose diet 24 h (n = 3)	NS	NS	NS	NS	NS	<0.05	<0.05	<0.005	NS	NS

* Mean sucrase-specific activities for normalized villus height (Fig. 3) have been compared among the diet groups noted.

significantly higher sucrase-specific activity in the villus tip region (70–100%) (Table I).

Fig. 4 compares the villus gradients of sucrase-specific activity in the animals fed CHO-free diet alone or after 24 h of a sucrose diet. As in the studies of the fasted animals, there was a rise in sucrase-specific activity only in the lower villus region. Indeed, as shown in Table I, only the sucrase-specific activity in the region from 30 to 50% of villus height was significantly higher in the 24-h sucrose diet group than in the CHO-free diet group. This peak of sucrase activity coincided with the leading region of radioactivity at 24 h (55±10%).

To demonstrate that the effects of sucrose feeding do not merely reflect a change in protein content relative to sucrase, the results were also calculated as total activity per segment. The absolute values of sucrase activity per segment were proportional to the size of the original cryostat sample, which varied among the experiments. However, the gradients of total sucrase activity paralleled those for sucrase-specific activity and demonstrated a similar elevation in lower villus activity after 24 h of sucrose diet, irrespective of pretreatment (fasted or CHO-free diet groups). This confirmed the presence of a new region of maximal sucrase activity at the lower villus in these animals.

Sucrose feeding for 24 h after the CHO-free diet produced changes in activity gradients for palatinase and maltase that paralleled those for sucrase, with the maximal activity in the lower villus region. A statistical analysis of the data obtained at the lower villus region is shown in Table II. A marked and highly significant increase was found in all three enzymes: a 3-fold rise in sucrase activity, a 10-fold increase in palatinase activity, and a 3-fold rise in maltase activity. Sucrose feeding produced no change in the villus gradients of lactase activity.

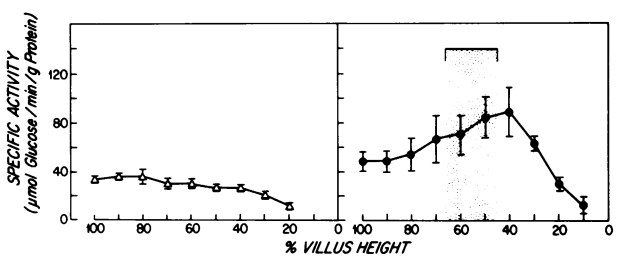

FIGURE 4 Comparison of the gradient of mean sucrase-specific activity for rats fed CHO-free diet for 3 d and then fed sucrose diet for 24 h. Methods and notations as in Fig. 3. The stippled bar is the leading region of radioactivity in the sucrose fed animals.

DISCUSSION

From indirect evidence, previous studies have suggested that adaptive changes in the activities of the jejunal brush-border disaccharidases might be mediated at the level of the crypt epithelial cell (4). Direct evidence for this hypothesis can be found in this study. Animals fed sucrose diets for up to 18 h after a fast showed no change in whole mucosal sucrase activity or

TABLE II

Statistical Analysis of the Effect of Sucrose Feeding on Sucrase, Palatinase and Maltase Specific Activities in the Lower Villus Region

Diet	Enzyme specific activity		
	Sucrase	Palatinase	Maltase
		μmol glucose/min/g protein	
CHO-free (n = 4)	23±2	2.5±0.3	105±18
+Sucrose (n = 3)	77±8*	24.0±2.1*	370±26*

Enzyme activity was measured in the region of the lower villus as described in Methods. Values are Mean±SE.
* $P < 0.001$ compared to CHO-free.

in the villus sucrase activity gradient. However, after 24 h of sucrose diet, there was a two- to threefold rise in whole mucosal sucrase activity, which appeared to result from a simultaneous change in the sucrase activity gradient characterized by a dramatic and significant rise in the lower villus region. Comparing these patterns to the measurement of cell migration rates revealed that the new area of maximal sucrase activity occurred in cells that had migrated from the crypt during the time period studied. Therefore, the villus epithelial cells with increased sucrase activity at 24 h must have been immature crypt cells at the onset of sucrose feeding. The epithelial cells that were mature villus cells at the onset of feeding showed no change in sucrase activity at 24 h. However, with continued sucrose feeding, epithelial cells nearer to the villus tip began to acquire increased sucrase activity. Indeed, by 36 h, the time required for nearly complete cell migration from crypt to villus tip (7), the entire villus appeared to have been replaced by epithelial cells with increased sucrase activity. Furthermore, as long as sucrose diet was continued beyond this period (up to 7 d) sucrase activity gradients demonstrated persistently elevated activity at the tip region.

These findings contrast strikingly with our own data (Fig. 1) and those of others (6) showing that when animals are fed standard laboratory chow diet, sucrase activity declines at the villus tip region. Because the carbohydrate in the chow diet consists mainly of starches at lower concentration than the sucrose of the test diet in this study, it is likely that substrate-dependent effects are responsible for the differences (2). Indeed, when fasted animals are fed a maltose diet, a rise in whole mucosal sucrase activity occurs but is not as dramatic as the rise seen with similar animals fed sucrose diet (2, 12).

Because of the well-known influence of fasting on intestinal mucosa (even when adequate nutrition is provided intravenously) (13–15) it was important to ascertain that the changes in sucrase activity seen in the animals fed sucrose diet after fasting were specific effects of the disaccharide. This was accomplished by repeating the experiments with animals stabilized on a CHO-free diet and then fed the isocaloric sucrose diet. After 24 h of sucrose feeding, the sucrase activity of whole jejunal mucosa was increased threefold over the level in control animals, while in the villus gradients, there was a coincident marked rise in sucrase activity in the lower villus region (Fig. 4, Table I). Thus, elevation in sucrase activity stimulated by sucrose is a specific effect of the disaccharide and not the result of mere nutritional intervention.

Two potential technical problems require further comment. First, the cryostat technique of Nordström et al. (6) isolates serial levels of intestinal mucosa from villus tip to crypt base and permits reproducible correlation of epithelial cell enzyme activity with histological documentation of the position of the cell. The method requires well-oriented, flat, cryostat samples; however, there remains a degree of intrinsic variability in the heights of adjacent villi that results in some mixing of surface epithelial cells not identical in maturation. Thus, sharp changes in enzyme activity gradients from villus tip to crypt base tend to be blunted by this technique. Nevertheless, as shown in this study, remarkably similar gradients were obtained from different animals within each diet group as shown by the standard error of the data in Figs. 1, 3, and 4.

Second, active DNA synthesis in the epithelial cells occurs only in the crypt proliferative zone (16); thus, radioactive thymidine is incorporated into DNA only at this level and remains with the cell throughout migration. The leading edge of radioactivity identifies cells that were in the synthetic phase of the cell cycle at the time of injection of the label. In this study, the leading edge of radioactivity was detected by scintillation counting of cryostat sections. This allowed a direct correlation of changes in enzyme activity with cell migration. Indeed, maximal regions of enzyme activity were always coincident with or just behind the leading edge of radioactivity, and were always first localized at the lower villus.

It is of interest that the mechanism by which epithelial cells increase sucrase activity in response to sucrose feeding has not been elucidated. Deren et al. (2) have demonstrated a sucrose-dependent increase in the V_{max} of sucrase activity suggesting accumulation of enzyme (2), but these workers have not provided information regarding the mechanism for this accumulation. Preliminary data from Das and Gray (17) indicated that sucrose might provide substrate stabilization of sucrase enzyme and thereby slow its degradation, but these observations have not yet been confirmed. As the crypt epithelial cells are known to contain enzymatically inactive sucrase precursor (18–20), it is possible to speculate that adaptive changes to dietary carbohydrate could occur through increased synthesis of precursor protein in the crpyt cells, increased conversion from inactive to active enzyme, decreased rates of degradation of either active or inactive enzyme or substrate-induced alterations in availability of enzymatic sites in the brushborder membrane. No direct evidence for any of these pathways is presently available. Nevertheless, these data do show that changes in sucrase activity in response to sucrose feeding are mediated at the level of the crypt epithelial cells, despite the fact that these cells are themselves devoid of sucrase enzyme activity.

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