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EFFECT OF CARBOHYDRATE INTAKE ON RAT SMALL INTESTINAL DISACCHARIDASE ACTIVITIES, WITH SPECIAL RESPECT TO DIURNAL RHYTHM

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EFFECT OF CARBOHYDRATE INTAKE ON RAT SMALL INTESTINAL DISACCHARIDASE ACTIVITIES, WITH SPECIAL RESPECT TO DIURNAL RHYTHM

by

Betty Kumiko Samulitis

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Oleg Koldovsky — 12/15/86

O. KOLDOVSKY
Professor of Pediatrics
and Physiology

DATE

DEDICATION

This thesis is dedicated to my parents, James and Kumi Samulitis, who have always supported and encouraged me through all of my endeavors.

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ABSTRACT

The mechanism(s) which control small intestinal disaccharidase activities are still unclear, therefore, it is unknown whether the dietary adaptation of disaccharidase activities and the diurnal fluctuations of disaccharidase activities rely on similar or independent mechanisms. When adult rats are switched from a low starch to an isocaloric moderate sucrose diet for 6 or 12 hours, an identical rise in each disaccharidase (sucrase, isomaltase, lactase, maltase, and trehalase) activity was seen, regardless of whether disaccharidase activities were rising or falling when the sucrose diet was given. Although the dietary adaptation of disaccharidase activities was independent of the time of day, determination of immunoreactive amounts of sucrase and lactase revealed that the diurnal fluctuations and the "short term" dietary adaptation of disaccharidase activities were both accompanied by corresponding changes in the amount of enzyme protein, whereas the "long term" dietary adaptation of disaccharidase activities also involved some (in)activation of the enzyme.

INTRODUCTION AND LITERATURE REVIEW

A number of enzymes that hydrolyze di- and oligosaccharides are present in the small intestinal brush border membrane of mammals. In rodents, these glycosidases are found in what are known as "multi-enzyme complexes" (Semenza, 1981; 1985b). A short review of these multi-enzyme complexes, and their substrate specificities is presented here.

To date, four disaccharidases or disaccharidase complexes have been identified (Semenza, 1985b): the **sucrase-isomaltase** complex consists of two subunits, sucrase and isomaltase. Sucrase is responsible for the hydrolysis of sucrose, while isomaltase is responsible for greater than 95% of isomaltase activity. Because both sucrase and isomaltase also hydrolyze maltose, some maltase activity is derived from sucrase-isomaltase. Thus, sucrase-isomaltase is important not only for the hydrolysis of sucrose but also in the final stages of starch digestion.

The **maltase-glucoamylase** complex (referred to as glucoamylase) includes two subunits, and is responsible for the hydrolysis of glucose oligomers of various lengths and structures which result from the process of starch digestion. Although it is not known if these two subunits

are identical, it is known that this complex possesses some isomaltase activity.

Another α-glucosidase is trehalase; it is believed that there is only one trehalase, and that its only substrates are α,α-trehalose and its 6-deoxy analog. Although trehalase has been purified recently (Nakano and Sacktor, 1985), little is known about its biochemical properties.

The β-glycosidase complex, lactase, is composed of two subunits. Two different catalytic sites are known to be present on this β-glycosidase complex, but whether each catalytic site is located in different subunits is not known. One catalytic site hydrolyzes lactose, and the other catalytic site hydrolyzes phlorizin and numerous aryl-β-glycosidases (i.e. cerebrosides which are present in milk).

All of these small intestinal disaccharidases are classified as "integral" membrane glycoproteins; they cannot be brought into solution by "normal" methods (homogenization in buffers of low or high ionic strength, EDTA, or sodium iodide). Therefore, solubilization of these disaccharidases is usually carried out by one of these methods (Semenza, 1981): (1) limited hydrolysis using various proteases, most commonly, papain; (2) detergent treatment, primarly Triton X-100; (3) freezing and thawing; or (4) butanol treatment.

Biosynthesis And Degradation Of Disaccharidases

An outline for the biosynthesis and degradation of disaccharidases is generally accepted by investigators in this field. The sucrase-isomaltase complex is believed to be synthesized as a single, large polypeptide chain on membrane bound polysomes (ribosomes), moved to the Golgi membranes to be glycosylated, and finally split into its active subunits, sucrase and isomaltase, by pancreatic proteases acting on the luminal side of the brush border membrane. The transfer of sucrase-isomaltase from the (intracellular) site of synthesis to the brush border membrane has been reported to take approximately 60 minutes (Hauri et al., 1979). Glucoamylase and lactase are believed to be similarly synthesized and processed, with a small exception in the processing step of lactase, which is proteolytically cleaved by intracellular protease(s). Turnover of disaccharidases on the brush border is relatively quick - estimated to be 11.5 hours - and it has been suggested that pancreatic proteases play a role in the degradation process (Alpers and Tedesco, 1975).

Alpers and Tedesco (1975) propose that disaccharidases, which may be located on the luminal surface of the brush border membrane, turn over as a result of intraluminal "pancreatic factors". In this paper, they report elevations in sucrase, maltase and lactase activities

in rats with 95% pancreatectomy, and a simultaneous decrease in turnover rates of these disaccharidases. Interestingly, trehalase, alkaline phosphatase, and other brush border glycoprotein activities are not affected in the same manner. Arvanitakis and Olsen (1974) report increases in sucrase and maltase levels in patients with chronic pancreatitis, while Kwong et al. (1978) used a mouse model with exocrine pancreatic insufficiency to show that an absence of intraluminal pancreatic proteases results in increased sucrase, maltase and lactase activities in the proximal intestine. Riby and Kretchmer (1985) provided further confirmation of this idea by demonstrating a lower rate of sucrase degradation in rats whose pancreatic secretions were bypassed, but whose normal bile flow was allowed. In these rats, a concomitant increase in sucrase activity is seen when compared to control rats. These investigators noticed that this is true only in the villus region, i.e. sucrase activity is not affected in the crypt region, and therefore concluded that pancreatic proteases release enzymes from the microvillus membrane only at sites exposed to intraluminal contents. Thus, pancreatic proteases appear to play a role of not only catalyzing the transformation of single chain sucrase-isomaltase into the final sucrase-isomaltase, but also in initiating the first steps of its degradation (Sjostrom et al., 1980).

However, the specific pancreatic component(s) which might be responsible for the degradation of disaccharidases have not been identified. Seetharan et al. (1976) report different sensitivities of sucrase and trehalase to chymotrypsin, trypsin and elastase, while Alpers and Tedesco (1975) and Hauri et al. (1980) suggest that elastase may help regulate disaccharidase turnover because of its broad specificity. The different sensitivities of sucrase and trehalase to pancreatic and lysosomal proteases lead Seetharan et al. (1976) to suggest a bi-directional degradation of intestinal hydrolases, from both the luminal and lysosomal compartments; thus a different membrane location of sucrase and trehalase could account for different sensitivities witnessed. Since trehalase is not readily solubilized from the brush border membrane by pancreatic proteases, trehalase is thought to be more deeply imbedded into the lipid bilayer of the brush border membrane.

Riby and Kretchmer (1985) speculate that brush border enzymes are degraded by different processes. The first, a "nondiscriminate" degradation whereby all proteins of the microvilli are degraded by endocytosis of brush border membrane vesicles and subsequent lysosomal digestion; the second, a removal of only brush border enzymes exposed to luminal contents by pancreatic proteases. If this is the

case, dietary composition could be a major controller of disaccharidase activity by virtue of its effect on pancreatic secretions.

Dietary Adaptation Of Small Intestinal Disaccharidase Activities

Many factors may affect disaccharidase activities of the adult rat: nutritional status, hormones, age and sex, intestinal bacteria, route and time of feedings, osmolarity of diet, diarrhea, pancreatic secretions, and dietary composition. The reader is referred to Koldovsky (1981) for a comprehensive coverage of these factors. Only one of the major factors which affects disaccharidase activities, dietary carbohydrate, is considered here. This topic has been extensively studied, and the reader is again referred to Koldovsky (1981) for a review of earlier works in this area. Earlier studies are, at best, difficult to compare because of the use of different strains, ages and sex of rats, arbitrary naming of regions of small intestine, variations in dietary composition (quality, quantity, nutritional adequacy), nonconformity in preparing tissues (i.e. whole mucosa including intestinal wall versus scraped mucosa), use of supernatants of centrifuged homogenates versus total homogenates, use of fresh versus frozen tissues, and in general, variation in assay techniques and methods. More recent works have become a little more

standardized among laboratories and, therefore, easier to compare and contrast.

Dietary carbohydrate composition is thought to be the prime factor which influences disaccharidase activity in adult rats. Earlier investigations using rats have concentrated on attempts to "induce" the activity of a specific disaccharidase by raising or maintaining the amount of its substrate in the diet (Bolin et al., 1969; Lifrak et al., 1976; Raul et al., 1978; Lebenthal et al., 1973). Although the activities of these disaccharidases appear to be stimulated by the presence of their own substrate in the diet, recent studies demonstrate that disaccharidase activities, in general, are altered as a result of a change in the quantity of carbohydrates in the diet (Bustamante et al., 1981; Yamada et al., 1981b; Goda et al., 1983).

When rats are switched from a low carbohydrate (5% of calories as starch or sucrose) to a high carbohydrate (70% of calories as starch or sucrose) diet, sucrase, lactase, and maltase activities in the proximal one-third of the jejuno-ileum of adult rats increase significantly (Bustamante et al., 1981; Koldovsky et al., 1983a; Koldovsky et al., 1982; Tsuboi et al., 1985). The reverse is also true; when adult rats are fed a high starch diet first, then switched to a low starch diet, a 40 - 80% drop in

sucrase, lactase and maltase activities is seen (Goda et al., 1983). Cezard et al. (1983) report increases in both sucrase and isomaltase activities in rats fed a 68% sucrose diet (compared to isocaloric carbohydrate free diets) while Riby and Kretchmer (1984) demonstrate a significant increase in jejunal sucrase activity of rats fed a 65% sucrose or 65% starch diet (compared to a 30% starch or carbohydrate free diet). The report by Yamada et al. (1981a) is especially supportive - 12 weeks old rats were initially fed a low starch (5% of calories) diet, then switched to an isocaloric moderate starch (40% of calories) or high starch (70% of calories) diet for 1, 2 or 3 days. A highly significant linear correlation was seen between dietary carbohydrate and both sucrase and lactase activities in the proximal jejuno-ileum, establishing the dependency of sucrase and lactase activities on the starch content of the diet. It would be interesting to see if maltase, isomaltase and trehalase activities respond similarly.

Furthermore, recent studies have demonstrated the lack of substrate specificity in the modification of disaccharidase activities by carbohydrate intake. For example, a change in the amount of dietary sucrose or starch (α-linkage) influences not only α-disaccharidase activities (e.g. sucrase), but also lactase activity, a

β-disaccharidase (McCarthy et al., 1980; Bustamante et al., 1981; Yamada et al., 1981a; Yamada et al., 1981b; Koldovsky et al., 1982; Koldovsky et al., 1983a; Leitcher et al., 1984; Goda et al., 1984; Goda et al., 1985d). Conversely, a change in the amount of dietary lactose influences not only lactase activity but also sucrase activity (Goda et al., 1984; Goda et al., 1985d).

Knowing that disaccharidase activities are capable of being modified by a change in diet, many questions arise. How and where are these changes manifested? What are the mechanisms responsible for these changes? First, one should understand that disaccharidases are expressed both longitudinally along the small intestine and columnarly along the villus-crypt units of the small intestine. The longitudinal distribution of disaccharidase activities are well established, with maximal activities found in the proximal and mid-jejunum and decreasing activities towards the ileum (Harrison and Webster, 1971). When dietary modification prompts a change in disaccharidase activities, maximal change in seen in the jejunum (Yamada et al., 1981a; Saito et al., 1975).

The general distribution of disaccharidase activities along the villus-crypt unit is also well established (Nordstrom et al., 1968; Nordstrom et al., 1969; Boyle et al., 1980a). Activities are not detected in the

crypt region, and increase as the enterocytes migrate toward the villus tips, a process which takes approximately two days in adult rats (Nordstrom et al., 1968). Interestingly, sucrase activity is expressed maximally in the middle villus, whereas lactase exhibits maximal activity at a more apical locus of the villus-crypt unit. Studies in piglets suggest increased cell migration rates of the intestinal epithelium during and after viral invasions, leading to an increased proportion of undifferentiated crypt-like cells (Kerzner et al., 1979), presumably "lactase-immature". Since minimal lactase activity is expressed in the crypts, this might explain the limited lactose hydrolysis exhibited during and after a bout of viral gastroenteritis.

Until recently, it was believed that the expression of disaccharidase activities in mature enterocytes was "pre-determined" while they were in the crypt region, and once the enterocytes matured and differentiated into the villus region, disaccharidase activities could not be altered. This is the opinion of Ulshen and Grand (1979) and Raul et al. (1980), who report an increase in sucrase activity in the crypt cells when a sucrose containing diet is given after a 24 hour fast. Both groups of investigators believe the changes in sucrase activity are initiated in the crypt region but are not expressed until these enterocytes mature and migrate toward the villus tip. However, other

investigators report that rats fed a low carbohydrate diet instead of fasted, then fed a high sucrose diet, show increases in sucrase and lactase activities along the entire villus-crypt unit within 18 to 24 hours (Yamada et al., 1981b; Koldovsky et al., 1983a). In other words, both immature and already mature enterocytes do respond to a change in dietary carbohydrate and express this "adjusted" disaccharidase activity during the present generation. The different conclusions were reached probably because of the "method" of lowering disaccharidase activities, i.e. low carbohydrate diet versus fasting. These two "methods" of lowering disaccharidase activities affect disaccharidase activities in a different manner; see page 13. The conclusions made by Ulshen and Grand (1979), and Raul et al. (1980) presumably did not take into account that the enterocytes had to first "recover" from the 24 hour fast before they could "adapt" to the new diet and express a new level of disaccharidase activity along the entire villus-crypt unit.

Determinations of cell migration rates, through the use of <3H>-thymidine injected simultaneously with presentation of a high sucrose diet, confirm that both immature and mature enterocytes, in rats previously fed a low carbohydrate diet, are capable of responding to a high

carbohydrate diet (Yamada et al., 1981b; Koldovsky et al., 1983a; Goda, et al., 1985d). In these studies, sucrase and lactase activities increase, although no change in cell migration rates are seen. When the diets are reversed, high starch diet first, then low starch diet - sucrase, lactase and maltase activities fall along the entire villus-crypt unit within 24 hours (Goda et al., 1983). These studies clearly show that mature enterocytes possess the ability to adjust their disaccharidase activities in response to dietary carbohydrate. This adaptation to dietary carbohydrate intake takes place almost immediately - much quicker than the lifespan of the enterocytes. The increases in sucrase activity (Cezard, et al., 1983; Goda et al., 1985d) and lactase activity (Goda et al., 1985d) occurs as early as three hours after sucrose feeding. Cezard et al. (1983) further report cytosolic sucrase, believed to be a putative precursor of the membrane enzyme, to be markedly elevated only two hours after sucrose feeding.

Although the dietary induced changes in disaccharidase activities occur along the entire villus-crypt unit, the mechanism of this change is still unresolved. One of the proposed mechanisms suggests that glucose has a nonspecific effect on both α- and β-disaccharidases, while fructose and galactose affects

either α- or β-disaccharidases, respectively (Bustamante et al., 1981; Yamada et al., 1981a). This proposal implies different mechanisms of adaptation among the disaccharidases, which would help explain the different effect of carbohydrate containing diets on sucrase and lactase activities along the villus-crypt unit; whereas sucrase activity is increased in the lower villus initially, the increase in lactase activity is seen at a more apical position on the villus-crypt unit (Goda et al., 1985d). This proposal would also help explain the different effect of starvation on sucrase and lactase activities (Yamada et al., 1983). Sucrase total activity progressively decreases during one, two or three days of starvation, but lactase total activity remains unchanged; after refeeding a high sucrose diet (72% of calories), sucrase total activity increases to starting values, while lactase total activity again does not change. Although these studies do not explain the mechanism of dietary adaptation of disaccharidase activities, they do suggest different process(es) for different disaccharidases.

Goda et al. (1985d) also suggest involvement of the glucose moiety in the adaptation of disaccharidase activities. These investigators report significiantly higher sucrase and lactase activities in rats fed a 40% sucrose or 40% lactose diet, when compared to a 40% starch

diet. Furthermore, whereas sucrase and lactase activities reach maximal levels within 24 hours on the sucrose or lactose diets, the starch diet takes two to three days to exhibit maximal disaccharidase levels. Similarly, Riby and Kretchmer (1984) show a 30% sucrose diet to be a more potent stimulator of sucrase activity than a 30% starch diet. It seems that the sucrose- and lactose-containing diets release glucose molecules more quickly than the starch-containing diet, increasing the glucose concentration in intestinal epithelial cells, and somehow accelerating the rise in sucrase and lactase activities.

Another mechanism for the adaptation of disaccharidase activities in response to a change in dietary carbohydrate has been suggested by Riby and Kretchmer (1984). These investigators suggest a "protective" effect of disaccharides, which might delay the degradation of disaccharidases by stabilizing the catalytic site of the enzyme.

An increased synthesis of inactive precursor protein or an increased conversion from inactive to active protein has also been proposed (Ulshen and Grand, 1979; Cezard et al., 1983). In studies comparing enzyme activity and immunoreactive protein, Cezard et al. (1983) estimate 80% of the increase in disaccharidase activity to be due to new enzyme synthesis and 20% due to enzymatic activation. Other

investigators report a constant ratio of enzyme activity and immunoreactive protein, suggesting no activation process is involved (Hauri et al., 1980; Skovbjerg, 1981; Goda et al., 1984; Goda et al., 1985a; Tsuboi et al., 1985).

Since carbohydrases contain 9 - 30% structural carbohydrate (Tsuboi et al., 1985), the glycosylation process of these disaccharidases might be affected by the amount of carbohydrate ingested. Kwong et al. (1982) report sucrase-isomaltase and maltase (but not lactase, which has the least amount of structural carbohydrate) activities increase approximately 10-fold when rats are switched from a low starch (5% calories) to a high starch (70% calories) diet. This suggests that there may be some relationship between dietary carbohydrate and the glycoprotein nature of the disaccharidases. However, it is not known whether the extent of glycosylation modifies the catalytic activity of the disaccharidases or whether the glycosylation process alters the susceptibility of the disaccharidases to proteolysis (e.g. by lysosoml proteases in the intracellular fractions, or by pancreatic proteases on the brush border membranes). These possibilities must be investigated in a future study.

Circadian Rhythms Of Small Intestinal Disaccharidase Activities

Circadian rhythms, in general, have been studied quite extensively, and the circadian rhythms of small intestinal disaccharidases are no exception. Circadian rhythmicities of rat small intestinal sucrase, lactase, isomaltase, maltase and trehalase activities have been reported by many investigators (Stevenson et al., 1975; Saito, et al., 1975; Saito et al., 1976a; Saito et al., 1976b; Nishida et al., 1978; George et al., 1985) in rats fed a standard non-purified diet ad libitum under "normal" night-day (12 - 14 hours of light per day) conditions. These investigators agree that the activities of these disaccharidases show a monophasic pattern with a peak around midnight and nadir approximately 12 hours later. Although a circadian variation is reported for most disaccharidases along the entire length of the small intestine, the amplitude of the fluctuation is greatest in the jejunum and less marked or not detected in the ileum. (Saito et al., 1975).

With unrestricted access to food and "normal" night-day lighting, disaccharidase activities begin rising about the time lights are turned off, coinciding with the usual nocturnal feeding habits of rats (Saito et al., 1975). Disaccharidase activities slowly increase to a peak which

occurs at midnight (± 3 hours), then gradually fall to a low, which occurs around noon, at a time when little eating takes place.

In contrast to normal feeding and lighting conditions, restricting food intake, altering lighting conditions or combining a restricted food intake and altered lighting conditions causes recognizable changes in the circadian variation of disaccharidase activities. When feeding is restricted to only four hours per day, whether "normal" lighting or continuous illumination is provided, peak disaccharidase activity shifts to just before or during feeding (Stevenson et al., 1975; Stevenson et al., 1976; Nishida et al., 1978; George et al., 1985). This was confirmed using 6-hour feedings every 24 hours with "normal" lighting with similar results (Saito et al., 1976a; Saito et al., 1976b). In rats fed for three hours every 12 hours under a constant light source, a 12-hour cycle of sucrase, isomaltase, maltase and trehalase and perhaps lactase is induced (Furuya et al., 1979). Under these conditions, the highest levels of disaccharidase activities are seen at the end of each meal.

Under continuous illumination, nocturnal eating and drinking patterns are abolished within 6 - 10 days (Siegel, 1961; Zucker, 1971). After four weeks on a constant lighting and ad libitum feeding regime, no daily

rhythms of food consumption, sucrase activity or maltase activity are seen (Nishida et al., 1978). But after four weeks of continuous darkness with free access to food, both food consumption and disaccharidase activities maintained daily fluctuations similar to those in rats kept under a "normal" lighting schedule (Nishida et al., 1978). Thus, considerable evidence points to the timing of food intake, rather than lighting conditions, as the primary synchronizer of disaccharidase activities (Stevenson et al., 1975; Saito et al., 1975; Nishida et al., 1978).

Some investigators (Bolles and Stokes, 1965; Stevenson and Fierstein, 1976; Saito et al., 1976a; Saito et al., 1976b) have suggested that the anticipation of feeding to be the trigger that cues the rise in disaccharidase activity in the evening hours, rather than the food intake itself. The studies by Saito et al. seem to support this hypothesis, demonstrating that established rhythms of maltase and sucrase activities persist for at least two days even when rats were starved (Saito et al., 1976a; Saito et al., 1976b).

The factors responsible for the daily variation of disaccharidase activities are still unclear. The immunoprecipitation experiments of Kaufman et al. (1980) indicate that the diurnal fluctuation in sucrase activity is

accompanied by a change in absolute amount of sucrase-isomaltase protein and is not a change in the enzymes' catalytic efficiency, and thus not a change in the proportion of active to inactive enzyme.

Kaufman and co-workers further used a radioactively labeled precursor of sucrase-isomaltase protein, <14C>-leucine, and found a clear circadian rhythm of enzyme degradation, but no difference in rate of <14C> - leucine incorporation (at two time points) into the protein. Although this suggests there is no fluctuation in the rate of enzyme synthesis, this should only be taken as supportive evidence against a circadian rhythm of enzyme synthesis, since <14C>-leucine incorporation was only tested at two time points. To either confirm or rule out the possibility of a circadian rhythm of enzyme synthesis, <14C> - leucine incorporation at several time points over 24 hours must be investigated.

If a diurnal variation in degradation rate does occur, the question arises - how does it occur? Because pancreatic enzymes have been implicated as a factor in the degradation of disaccharidases, investigators have compared the circadian rhythm of disaccharidases to the daily fluctuation in pancreatic enzyme contents. No cyclic pattern of pancreatic trypsinogen content is seen (Girand-Glober et al., 1980; George et al., 1985), but pancreatic

amylase, lipase and chymotrypsinogen content do follow a daily cyclic pattern (Girand-Glober et al., 1980). The possible involvement of all pancreatic proteases in the lumen of the small intestine should be investigated in a future study.

Another factor suggested in the expression of a circadian rhythm of disaccharidase activity is the presence of the diet in the lumen. Saito et al. (1978) tested this by comparing sucrase and maltase activities in "isolated" (no direct contact with diet) segments of jejunum which receive normal nerve and blood supply, with "control" segments (normal contact with diet and normal nerve and blood supply). Although the disaccharidase activities are slightly lower in the "isolated segments" than in the control segments, similar circadian changes are seen in both segments. Saito et al. (1978) also report that sucrase and maltase activities remain, even in rats fed a carbohydrate-free diet (lactase and trehalase activities were not investigated). Thus, actual physical contact between diet (dietary carbohydrate) and the mucosa does not seem to play an essential role in the circadian rhythmicity of sucrase and maltase activities.

From reviewing the literature, it is evident that disaccharidase activities are influenced by dietary carbohydrate and that a circadian rhythm of disaccharidase activity is present. Because the precise mechanisms by which disaccharidase activities are controlled are yet unknown, whether the dietary adaptation of disaccharidase activities and the diurnal variation of disaccharidase activities rely on similar or independent mechanisms is still speculation.

The objective of this thesis was to determine whether the "short term" dietary adaptation of disaccharidases depends on the normal rhythmicities of disaccharidase activities, i.e. is the dietary adaptation of disaccharidase activities different at a time of increasing or decreasing disaccharidase activity. (The "short term" dietary adaptation, which implies the "first response", is in contrast to the "long term" dietary adaptation, which implies a new steady state is reached). In this way, it was hoped that it could be determined if the dietary adaptation of disaccharidase activities and the diurnal fluctuations in disaccharidase activities are the result of similar or different mechanisms.

The diurnal pattern of disaccharidase activities was first established in Experiments 1 and 2. Then, in Experiments 3 and 4, the "short term" adaptation of

disaccharidase activities to dietary carbohydrate at selected time periods of the day was examined.

MATERIALS AND METHODS

Experiment 1

This pilot study was conducted to establish the presence of a diurnal fluctuation in disaccharidase activities, and to monitor food intake to use as a basis for later studies. Six week old female Sprague-Dawley rats were received from Harlan Sprague-Dawley (Indianapolis, IN), and allowed to become acclimated to the new environment for seven days. During those seven days, the rats were fed a standard non-purified diet (Wayne Lab Blox, Allied Mills, Chicago, IL) ad libitum. For composition of Lab Blox, see Appendix A. At seven weeks of age, the rats were divided into two groups of approximately equivalent weights. One group (n = 14) was fed a purified low starch (LST) diet (5% of calories as starch, 21% protein, 73% fat) and the other group (n = 14) an isocaloric high starch (HST) diet (70% of calories as starch, 21% protein, 7% fat) for 14 days. For composition of diets, see Table 1. From this time on, rats were housed individually in wire bottom cages so that food intake could be closely monitored. Water was freely available throughout the entire experiment. A "normal" light cycle, with 12 hours of darkness (6pm - 6am) was maintained.

Table 1. Composition of Diets

INGREDIENT	LOW STARCH		HIGH STARCH		SUCROSE	
	% WT	% CAL	% WT	% CAL	% WT	% CAL
Casein	15.90	20.80	15.70	20.91	16.60	21.60
Corn starch	3.60	4.71	52.60	70.06	-	-
Sucrose	-	-	-	-	30.80	40.08
Corn Oil	24.70	72.69	2.40	7.19	12.50	36.60
AIN Mineral Mix	2.80	0.43	2.80	0.43	2.80	0.43
AIN Vitamin Mix	0.80	1.06	0.80	1.06	0.80	1.06
DL-Methionine	0.24	0.33	0.24	0.33	0.24	0.33
Choline Bitartrate	0.16	0.00	0.16	0.00	0.16	0.00
2% Agar Soln, ml	51.80	0.00	25.30	0.00	36.10	0.00

AIN Mineral Mix contains 11.8g sucrose/100 g (manufacturer literature);
AIN Vitamin Mix contains 97.3g sucrose/100 g (manufacturer literature);
all values per 100 g diet

LST and HST diets were fed ad libitum, with fresh diet prepared and given every other day. Food consumption was measured every two days by weighing the diet remaining in the feed pan and correcting for spillage. On the 7th and 11th days, food intake was measured every three hours, for 24 hours. Individual body weights were recorded every other day and at the time of sacrifice.

The rats were sacrificed by decapitation at 9am, 12pm, 3pm, 9pm, 12am, and 3am in a fed state. At each time point, two rats from each dietary group were sacrificed, with the exception of the 12pm and 12am time points, when three rats per dietary group were sacrificed (Figure 1). All rats within one time point were sacrificed within 30 minutes.

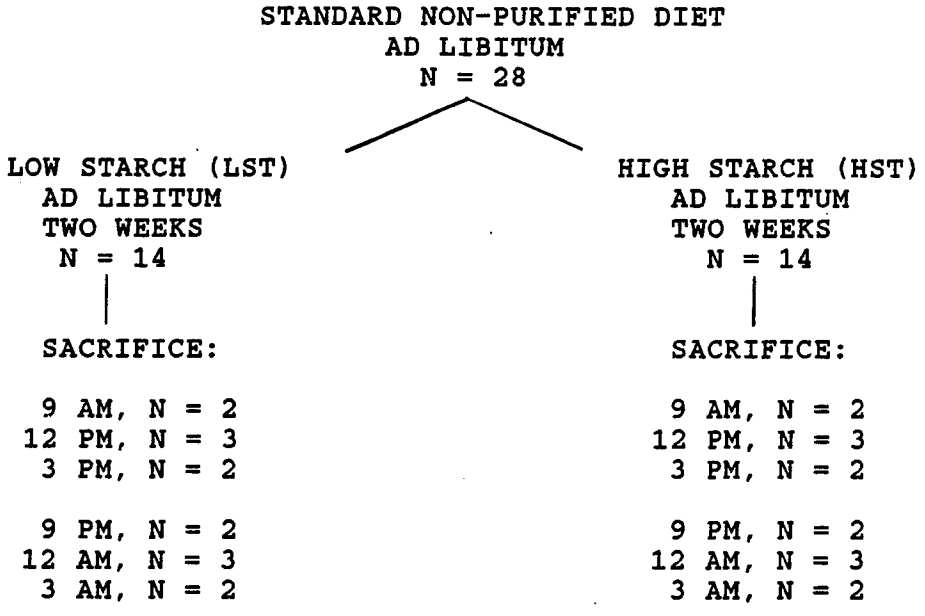


Figure 1. Scheme of Experiment 1

Experiment 2

A second experiment was conducted to verify the diurnal trend of disaccharidase activities seen in Experiment 1, and was carried out using a similar design. Sixteen rats were raised until six weeks of age, as described above. At six weeks, eight rats were fed the low starch diet and the remaining eight were fed the high starch diet, both ad libitum. After seven days on the purified diets, half of the rats from each dietary group were sacrificed between 9 and 10am and the remaining half betweeen 9 and 10pm (Figure 2).

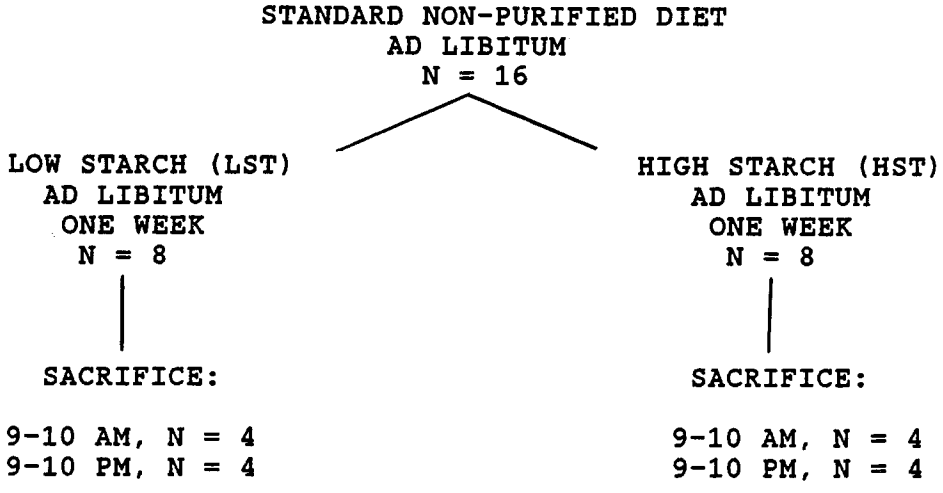


Figure 2. Scheme of Experiment 2

Experiment 3

Experiments 3 and 4 were conducted to study the "quick" response of disaccharidase activities (i.e."short term" adaptation) to a change in the amount of dietary carbohydrate for 6 hours (Exp 3) or for 12 hours (Exp 4). Twenty-four rats were raised, as described above, until seven weeks of age. They were then marked and housed four per cage. Sixteen rats were fed the low starch diet and eight rats were fed an isocaloric sucrose (SUC) diet (40% of calories as sucrose, 22% protein, 37% fat). See Table 1 for composition of diets. These diets were given ad libitum for seven days, with new diet given every other day.

After seven days, the rats fed the LST diet were fasted for four hours prior to force feeding, which was necessary to control caloric intake between dietary groups. Rats fed the SUC diet remained on the ad libitum feeding schedule until sacrifice. One half of the rats receiving the LST diet were force fed a "liquified" low starch diet (LST/LST) which was similar in composition to the "solid" low starch diet they had been receiving; the remaining half received a "liquified" sucrose diet (LST/SUC) with similar composition to the "solid" sucrose diet (Figure 3). Composition of the "liquified" diets were essentially the same as the "solid" diets, except the agar was eliminated, and double distilled water was used to dilute the diet to a

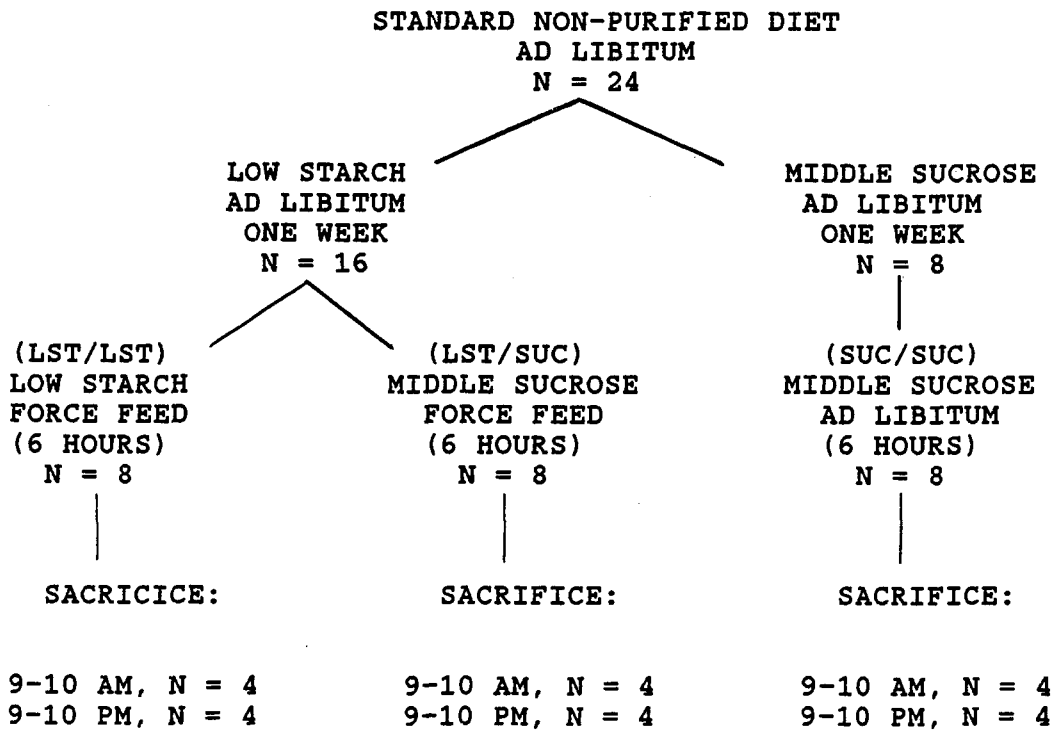


Figure 3. Scheme of Experiment 3

consistency which could pass through a feeding tube (Infant feeding tube, 8 Fr x 15", Mallinckrodt, Inc.). The rats were fed the "liquified" low starch and sucrose diets twice - 3 and 6 hours prior to sacrifice. Caloric density of the force feeding diets was approximately 2.4 kcal/ml. The rats were given approximately 7.5 kcal/100g body weight/6 hr (approximately 3 ml/rat/feeding), which corresponded to the amount of diet consumed (ad libitum) by rats of a similar age and body weight.

Rats were force fed at 3am and 6am for the "AM sacrifice" and at 3pm and 6pm for the "PM sacrifice". Force feeding was accomplished by passing the gavage feeding tube intragastrically and pushing calculated amounts of diet via a 12 ml syringe. All rats tolerated the force feeding procedure well. At 9am and 9pm, four rats from each dietary group, LST/LST, LST/SUC, and SUC/SUC, were sacrificed by decapitation.

Experiment 4

A fourth experiment, with similar design as the third, was carried out with the following changes: 16 rats were raised until nine weeks of age, as above. At that time, they were given the low starch diet, ad libitum, for one week. All rats were fasted for four hours, then force fed either a "liquified" low starch or sucrose diet for 12 hours. Three feedings were administered at 10pm, 2am, and 6am for the "AM sacrifice" and at 10am, 2pm, and 6pm for the "PM sacrifice". Each feeding provided approximately 6.7 kcal/100 g body weight/4 hours. A total of approximately 20 kcal/100 g body weight was given in the 12 hour feeding period. At 10am and 10pm, four animals from each dietary group, LST/LST and LST/SUC, were sacrificed (Figure 4).

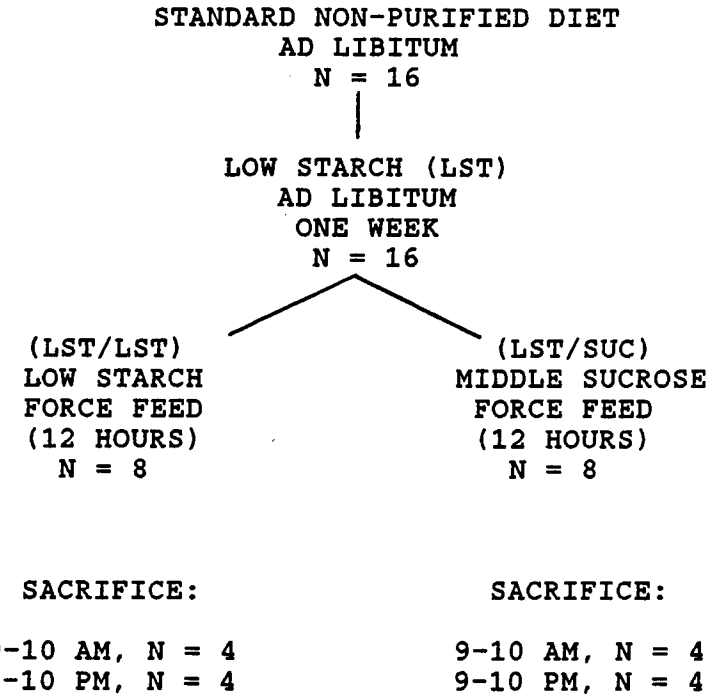


Figure 4. Scheme of Experiment 4

Preparation Of Samples

The jejuno-ileum was removed from the ligament of Treitz to the ileo-cecal valve and divided into three equal parts along its length. These three segments, from oral to anal, will hereafter be referred to as proximal, middle and distal jejuno-ileum. Each segment was flushed with 10 ml of ice cold saline and the flush was frozen at -20 °C. The mucosa of the proximal jejuno-ileum was scraped on ice using a glass slide and frozen at -20 °C until assay of disaccharidases.

For villus-crypt slicing, a 5mm X 5mm segment from the center of the proximal jejuno-ileum of each rat was removed, mounted flatly on a microscope slide, and quickly frozen using liquid nitrogen. Without thawing, the sample was protected with Tissue-Tek OCT Compound (Miles Scientific, Naperville, IL) and saran wrap and stored at -18 °C until slicing. To slice, the sample was attached (via frozen 1% agar block) to a cryostat chuck; with the cryostat setting on #5, 10 um thick slices were cut. Every first through ninth slice were combined and homogenized in double distilled water using a Vortex mixer. Disaccharidase activities and protein concentration were assayed as described below. Every tenth slice was stained and examined under a phase contrast microscope for "normal" appearance of villus and crypt cells. In this way, the start of villus

cells and the approximate area of villus-crypt junction could be revealed.

Enzymatic Assays

Thawed mucosa of the proximal jejuno-ileum (region of maximum disaccharidase activities) were homogenized with a Teflon homogenizer on ice in 4 volumes of 10 mM potassium phosphate buffer, pH 7.0, then diluted with double distilled water, as appropriate. Disaccharidase activities were assayed according to the method of Dahlqvist (1964), using glucose oxidase (Sigma Chemical Company, St. Louis, MO) in 0.5 M Tris-HCl buffer, pH 7.0. Sucrose, palatinose, lactose, maltose, and trehalose were used as substrates for sucrase, isomaltase, lactase, maltase, and trehalase, respectively. The substrate concentration in the assay mixture was 28 mM. All substrate solutions were prepared in 0.1 M sodium maleate buffer, pH 5.8. Lactose substrate contained p-hydroxy-mercuribenzoic acid (Calbiochem, La Jolla, CA) to inhibit residual lysosomal acid-β-galactosidase activity (Koldovsky et al., 1969). All determinations were made under the condition of linearity of time and concentration of enzyme. Protein was assayed according to Lowry (1951) using bovine serum albumin (Fraction V, U.S. Biochemical Corporation, Cleveland, OH) as standard.

Data were expressed as specific activity (μmoles substrate hydrolyzed per hour per milligram protein), except in cases where protein content per (proximal jejuno-ileal) segment varied between dietary groups, in which case results were also expressed as total activity (μmoles substrate hydrolyzed per hour) per small intestinal segment.

Trypsin Assay

Trypsin activity in the intestinal flush was determined by a slight modification of the method of Erlanger et al. (1961). Briefly, the intestinal flush (see above) was thawed and homogenized with a Teflon homogenizer on ice. Dilution was 1:5 in the proximal jejuno-ileum, 1:10 in the middle jejuno-ileum, and 1:20 in the distal jejuno-ileum, with 50 mM potassium phosphate buffer, pH 7.0.

The diluted flush (0.2 ml) was pre-incubated with 20 μl of double distilled water or with an enterokinase solution (5 mg/ml; Sigma Chemical Company, St. Louis, MO) for 50 minutes at 37 °C in a shaking water bath. This was done to measure trypsin activity already present in the luminal flush and also to activate any remaining trypsinogen. One ml of 94 mM α-N-benzoyl DL-arginine-p-nitroanilide (BAPNA) was added and incubated (37 °C shaking water bath; 80 cycles per minute) for exactly 10 minutes. To stop the reaction, 0.2 ml of 0.2 N HCl was added. Tubes were brought to room temperature, and the color enhanced by

adding 0.2 ml each of 0.1% sodium nitrite, 0.5% ammonium sulfamate, and 0.05% naphthyl-ethylenediamine-dihydrochloride. Absorbance of the supernatant was read at 550 nm, and the amount of p-nitroanilide released was compared to the standard curve of p-nitroanilide in 0.1 M Tris buffer, pH 7.4. Data were expressed as µmoles p-nitroanilide released per min per ml.

Papain Solubilization Of Disaccharidases

Disaccharidases were solubilized by papain, as described by Goda et al. (1984a). Briefly, 0.6 ml of mucosal homogenate was incubated for 45 minutes in a 37 °C shaking water bath (80 cycles per minute) with 0.29 mg of papain (crude papain, Type II, Sigma Chemical Company, St. Louis, MO), which had been pre-incubated (15 minutes) with 0.72 mg of cysteine hydrochloride (Calbiochem, LaCholla CA). This papain-treated homogenate was dialyzed (Spectrophor, VWR Scientific Inc., 12000-14000 MW cut-off) for 16 hours against 10 mM potassium phosphate buffer, pH 7.0 (4 °C), and then centrifuged at 105,000g for 1 hour. Sucrase, isomaltase and lactase activities and protein concentration of both the supernatant and the precipitate were determined to monitor recovery.

Electroimmuno Assay

Immunoreactive amounts of sucrase-isomaltase (SI) and lactase (L) were measured by electroimmuno assay (rocket technique) of Laurell (1972). Anti-SI and anti-L serum and purified SI and L were provided by Dr. Toshinao Goda. A 1% agarose gel was prepared in 35 mM Tris barbital buffer, pH 8.8. SI plates contained anti-SI serum diluted 1:150; L plates contained anti-L serum diluted 1:300. Purified SI or L was used as standard at the following concentrations: SI = 10, 30, 50 and 100 µg/ml; L = 5, 10, 30 and 50 µg/ml.

Ten µl samples of the papain treated supernatants of mucosal homogenates were applied to the plates. When necessary, samples were diluted with 10 mM potassium phosphate buffer, pH 7.0. Electrophoresis was run for 18 hours (4 °C) at approximately 80 V (9-11 mAmp). When completed, the plates were rinsed with several changes of 0.9% saline to remove any unprecipitated proteins, carefully pressed with filter paper, and then allowed to dry at room temperature. A 0.025% Coomassie Brilliant Blue solution (in destaining solution, 9% acetic acid in 50% methanol) was used to stain the immunoprecipitates. As necessary, the plates were destained. The height of each peak was measured to the closest millimeter, and compared to the standard curve of purified SI and L, which was linear from 30 -100 µg/ml for SI and from 10 - 50 µg/ml for L.

Statistical Analysis

Data are expressed as mean ± sem. Statistical analysis was performed by Student's t-test and analysis of variance. The significance level chosen was $p < 0.05$.

RESULTS

Diurnal Rhythm Of Disaccharidase Activities

To establish the presence or absence of a diurnal rhythm of five small intestinal disaccharidase activities (sucrase, isomaltase, lactase, maltase and trehalase) in the proximal jejuno-ileum, rats were placed on either a low starch (LST) or high starch (HST) diet ad libitum for two weeks, then sacrificed at six time points (9am, 12pm, 3pm, 9pm, 12am, and 3am) within one 24 hour period (Experiment 1; See Figure 1). Although rats on both diets initially lost weight when fed the new diets, both dietary groups progressed with normal and similar weight gain (Table 2) from the third day on.

The food intake over a 24 hour period was recorded to determine the pattern of ad libitum intake throughout the day and to compare the intakes of the two dietary groups. Except for the three hour time period between 9pm and 12am, rats of both dietary groups consumed comparable amounts throughout the 24 hours (Figure 5). During this one three hour time period, the rats fed the low starch diet consumed slightly (28%) less than the rats fed the high starch diet ($p < 0.05$), but there was no significant difference in total caloric intake/100 g body weight over the 24 hour period

Table 2. Summary of body weights and small intestinal protein content of rats on ad libitum low starch (LST) or high starch (HST) diet for two weeks (Exp 1)

DIET	BW, DAY 0(a)	BW, DAY 13(b)	PRO/SEG(c)
LST	175.5 ± 2.5	193.4 ± 3.2	149.2 ± 5.9
HST	175.4 ± 3.2	191.7 ± 4.7	127.6 ± 5.5 #

(a) = body weight at beginning of experiment, grams
(b) = body weight one day before sacrifice, grams
(c) = protein content per small intestinal segment, mg (proximal jejuno-ileum)

values represent mean ± sem for n = 14
significantly different from LST; $p < 0.05$

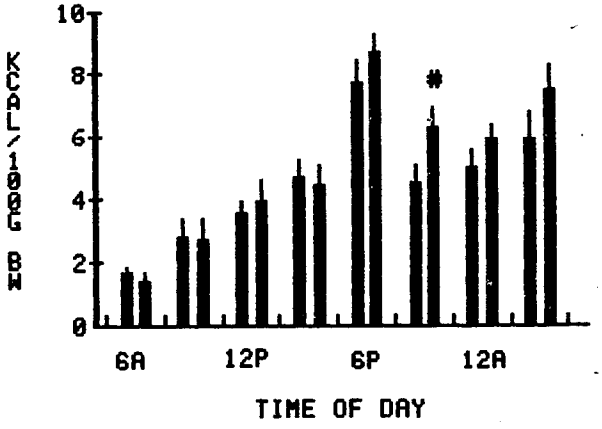


Figure 5. Comparison of food intake per three hours of rats on ad libitum low starch or high starch diet (Exp 1)

left bar = low starch diet; right bar = high starch diet; values represent mean ± sem for n = 13-14

signif diff from LST, $p < 0.05$

(34.9 ± 1.8 kcal/100 g body weight per 24 hours in LST; 40.0 ± 2.2 kcal/100 g body weight per 24 hours in HST). Being nocturnal animals, rats in both dietary groups consumed considerably more calories ($p < 0.001$), during the dark hours of 6pm - 6am (23.1 ± 1.4 kcal/100 g body weight in LST; 27.3 ± 0.9 kcal/100 g body weight in HST) than during the daytime, 6am - 6pm (11.8 ± 1.2 kcal/100 g body weight in LST; 12.8 ± 1.9 kcal/100 g body weight in HST).

Mucosal protein concentration, when expressed as mg per wet weight of mucosa, did not differ significantly between the two dietary groups. But when expressed as protein content per proximal jejuno-ileal segment, rats fed the low starch diet had slightly (17%) higher total protein content per segment than rats fed the high starch diet ($p < 0.05$; Table 2). To avoid any possibility of an artifact arising from this difference in protein content, disaccharidase activities are shown in both specific (µmoles substrate hydrolyzed per hour per mg protein; Figure 6) and total (µmoles substrate hydrolyzed per hour per small intestinal segment; Figure 7) activities. However, the patterns of daily fluctuation in disaccharidase activities were essentially the same in both expressions.

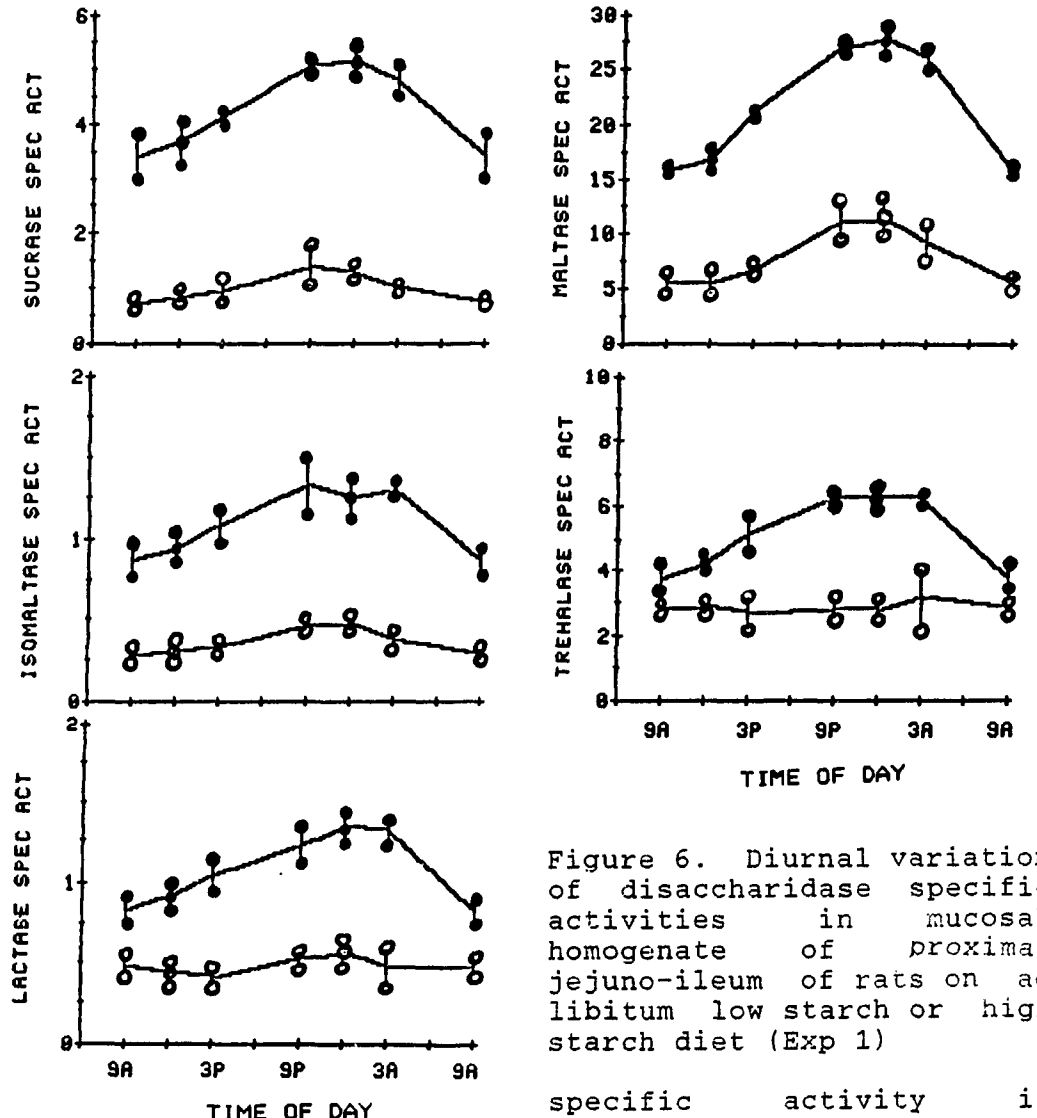


Figure 6. Diurnal variation of disaccharidase specific activities in mucosal homogenate of proximal jejuno-ileum of rats on ad libitum low starch or high starch diet (Exp 1)

specific activity is expressed as µmoles substrate hydrolyzed per hr per mg protein; individual values are shown by open circles (low starch) or by closed circles (high starch)

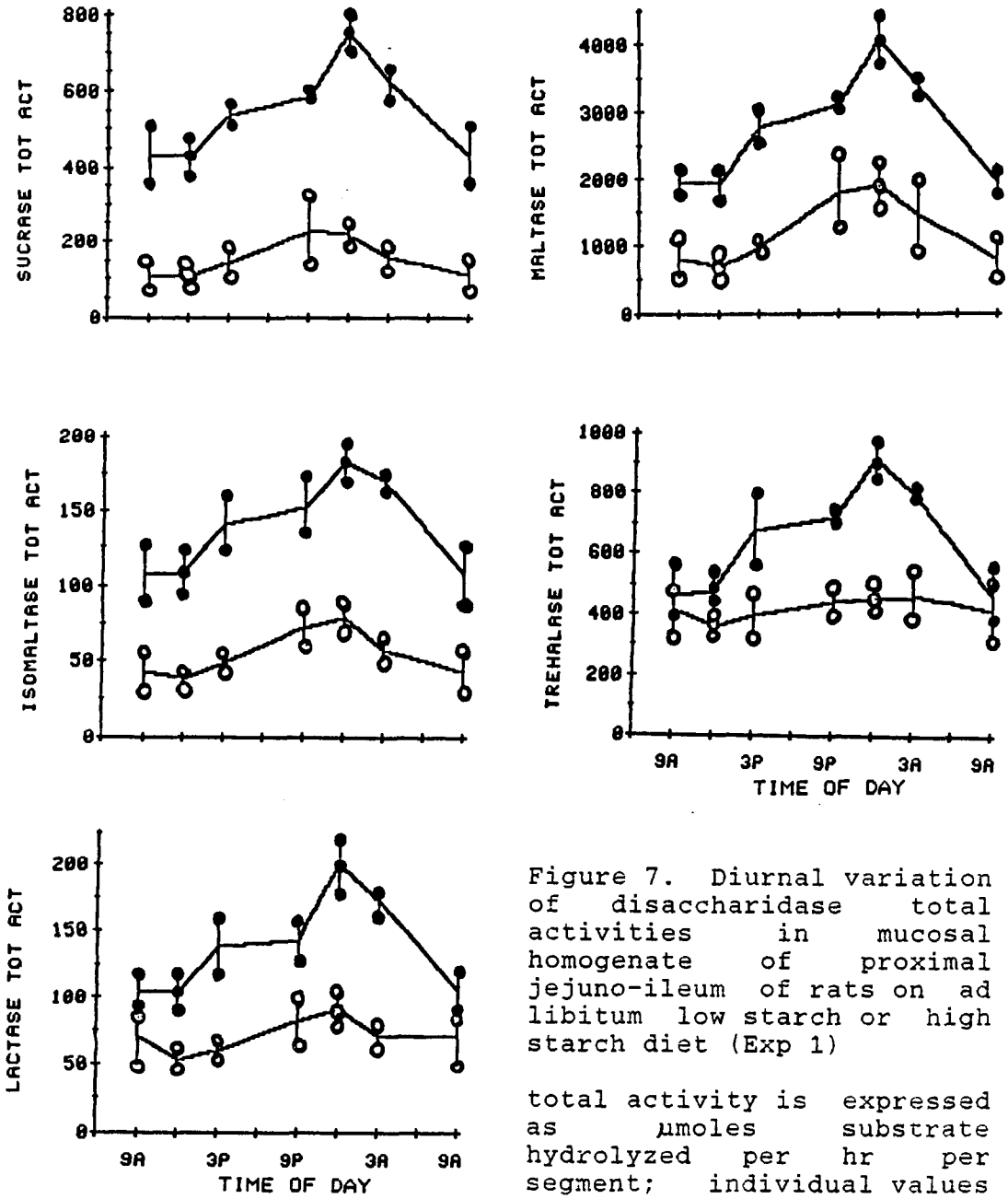


Figure 7. Diurnal variation of disaccharidase total activities in mucosal homogenate of proximal jejuno-ileum of rats on ad libitum low starch or high starch diet (Exp 1)

total activity is expressed as µmoles substrate hydrolyzed per hr per segment; individual values are shown by open circles (low starch) or by closed circles (high starch)

A cyclic pattern of increasing and decreasing disaccharidase activities was observed in both dietary groups (Figures 6 and 7), with a peak at night (9pm - 12am) and nadir approximately 12 hours later (9am - 12pm). This monophasic rhythm was seen in sucrase, isomaltase, lactase, maltase and trehalase activities in rats fed the high starch diet, as well as in sucrase, isomaltase, and maltase activities in rats fed the low starch diet. Lactase activity in rats on the low starch diet seemed to exhibit a diurnal tendency, although this was hard to establish conclusively, since the difference between the highest and lowest mean lactase activity was only 27% (specific activity). No diurnal tendency of trehalase activity was seen in rats fed the low starch diet.

The trypsin activity in the lumen of the proximal jejuno-ileum was examined for a diurnal pattern. Pre-incubation of the luminal flush with enterokinase produced only a slight (15 - 31%) increase in trypsin activity, and the percentage of increase was similar between the two dietary groups regardless of the time of sacrifice. Therefore, only values of trypsin activity determined without preincubation with enterokinase are shown. As shown in Figure 8, no detectable cyclic pattern of luminal trypsin activity was seen. Luminal trypsin activity in the

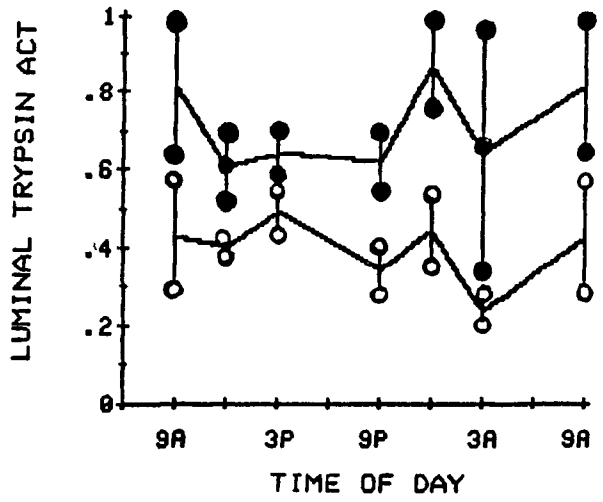


Figure 8. Diurnal variation in luminal trypsin activity in proximal jejuno-ileum of rats on ad libitum low starch or high starch diet (Exp 1)

trypsin activity is expressed as µmoles of p-nitroanilide produced per minute per ml; individual values are shown by open circles (low starch) or by closed circles (high starch)

middle jejuno-ileum showed similar fluctuations with no discernable trend that could be viewed as a diurnal rhythm (data not shown).

Because only two to three rats were sacrificed per time point per dietary group in this experiment, valid statistical analysis could not be performed. Therefore, to establish whether the differences in disaccharidase activities at the various time points were statistically significant, the above experiment was repeated, using a larger number of rats at two selected time periods, 9 - 10pm and 9 - 10am, the peak and nadir of disaccharidase activities, respectively (Experiment 2; See Figure 2).

In this experiment, eight rats were each fed a low starch or high starch diet ad libitum for one week. Four rats from each dietary group were sacrificed at 9 - 10am and the remaining four at 9 - 10pm (See Figure 2). Table 3 summarizes the body weights and small intestinal protein content of the two dietary groups; no significant difference was seen in either body weights or mucosal protein content between the two dietary groups. Since the protein content was similar between the two dietary groups, disaccharidase activities were expressed only as specific activity (μmoles substrate hydrolyzed per hour per mg protein).

With the exception of trehalase activity in rats fed the low starch diet, disaccharidase activities were

Table 3. Summary of body weights and small intestinal protein content of rats on ad libitum low starch (LST) or high starch (HST) diet for one week (Exp 2)

DIET	BW, DAY 0(a)	BW, DAY 6(b)	PRO/SEG(c)
LST	163.8 ± 2.7	176.8 ± 2.5	90.9 ± 2.2
HST	160.4 ± 2.2	175.5 ± 2.0	90.4 ± 5.0

(a) = body weight at beginning of experiment, grams
(b) = body weight one day before sacrifice, grams
(c) = protein content per small intestinal segment, mg (proximal jejuno-ileum)

values represent mean ± sem for n = 8

significantly elevated ($p < 0.01$) at night compared to corresponding disaccharidase activities in the morning, confirming the trend seen in the first experiment (Figure 9). Disaccharidase activities of rats fed the high starch diet were always higher than disaccharidase activities of rats fed the low starch diet at the same time period (except trehalase activity in the morning). Thus, a diurnal rhythm of sucrase, isomaltase, lactase, and maltase activities was maintained, regardless of dietary regime, whereas a diurnal rhythm of trehalase activity was maintained only in the high starch group.

Luminal trypsin activity of the proximal, middle and distal segments of the jejuno-ileum was compared between the two dietary groups and between the two time periods. As in the first experiment, no diurnal variation of luminal trypsin activity could be detected (Table 4), and no significant difference in luminal trypsin activity between dietary groups was seen (Table 4).

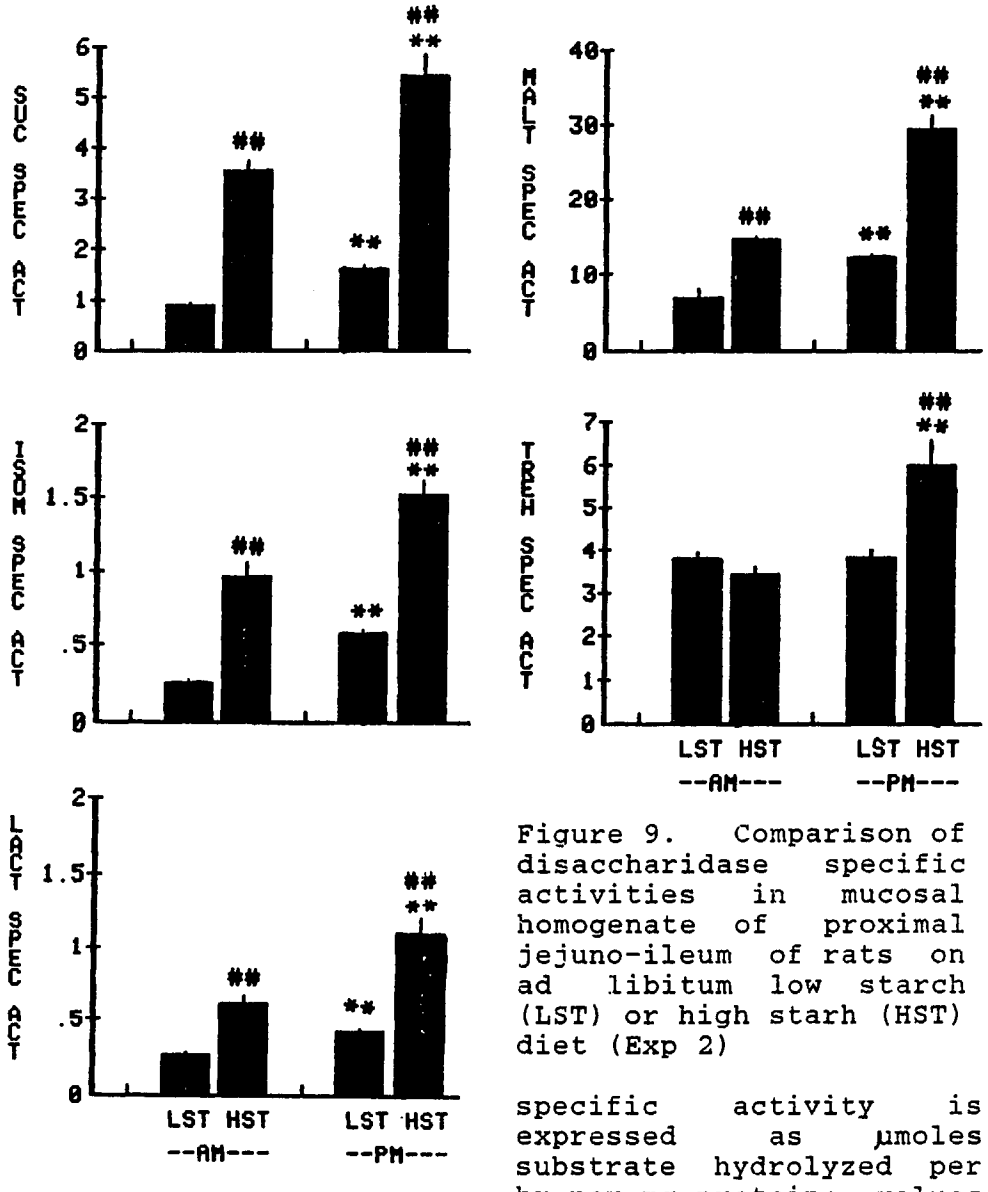


Figure 9. Comparison of disaccharidase specific activities in mucosal homogenate of proximal jejuno-ileum of rats on ad libitum low starch (LST) or high starh (HST) diet (Exp 2)

specific activity is expressed as μmoles substrate hydrolyzed per hr per mg protein; values represent mean ± sem for n=4;
*,** signif diff from AM;
#,## signif diff from LST;
$p < 0.05$ & $p < 0.01$, respectively

Table 4. Luminal trypsin activity in proximal, middle and distal jejuno-ileum of rats on ad libitum low starch (LST) or high starch (HST) diet for one week (Exp 2)

SEGMENT	LST/AM	HST/AM	LST/PM	HST/PM
PROX	0.46 ± .12	0.57 + .12	0.44 ± .11	0.74 ± .06
MIDDLE	1.03 ± .23	0.98 ± .30	1.15 ± .11	1.46 ± .15
DISTAL	1.20 ± .31	1.18 ± .28	1.33 ± .38	1.75 ± .36

trypsin activity is expressed as µmoles of p-nitroanilide produced per minute per ml; values represent mean ± sem for n = 4

To determine whether the diurnal variation of disaccharidase activities was due to an actual change in amount of enzyme protein, or was a result of a higher proportion of active to inactive enzyme, immunoreactive amounts of sucrase and lactase were determined in papain solubilized extracts prepared from mucosal homogenates of the proximal jejuno-ileum. To verify the efficacy of papain treatment, the recoveries of sucrase, isomaltase and lactase activities, and of protein, were checked in the papain supernatant. The recoveries of sucrase, isomaltase, and lactase activities (as compared to homogenate disaccharidase activities) were approximately 84%, 62%, and 76%, respectively, and were consistent between the dietary groups regardless of the time of sacrifice. The recovery of protein was also similar between the dietary groups, regardless of the time of sacrifice. Therefore, it was considered pertinent to compare the activities of sucrase and lactase with the amounts of corresponding immunoreactive enzyme proteins in the papain solubilized supernatant.

Table 5 shows the activity and immunoreactive amounts of sucrase and lactase in the papain supernatant of the proximal jejuno-ileum. In both dietary groups, not only sucrase and lactase activities (columns a and d), but also the amounts of immunoreactive sucrase and lactase (columns b and e), were always higher at night when compared to the

Table 5. Enzyme activity and immunoreactive amounts of sucrase and lactase in the papain supernatant of the proximal jejuno-ileum of rats on ad libitum low starch (LST) or high starch (HST) diet for one week (Exp 2)

	SA(a)	IRS(b)	S/IRS(c)
LST/AM	3.47 ± 0.14	4.48 ± 0.26	0.78 ± 0.02
HST/AM	10.59 ± 1.16##	10.22 ± 1.02##	1.03 ± 0.02##
LST/PM	6.70 ± 0.31**	7.98 ± 0.30**	0.84 ± 0.04
HST/PM	22.22 ±11.19##**	18.70 ± 0.94##**	1.19 ± 0.02##**

	LA(d)	IRL(e)	L/IRL(f)
LST/AM	0.97 ± 0.05	2.53 ± 0.10	0.39 ± 0.02
HST/AM	1.93 ± 0.18##	4.16 ± 0.34##	0.46 ± 0.01#
LST/PM	1.42 ± 0.04**	3.51 ± 0.10**	0.40 ± 0.01
HST/PM	3.71 ± 0.18##**	8.83 ± 0.36##**	0.42 ± 0.01

(a) = sucrase specific activity, µmoles substrate hydrolyzed per hour per mg protein
(b) = immunoreactive sucrase, µg/mg protein
(c) = sucrase activity/immunoreactive sucrase
(d) = lactase specific activity, µmoles sustrate hydrolyzed per hour per mg protein
(e) = immunoreative lactase, µg/mg protein
(f) = lactase activity/immunoreactive lactase

values represent mean ± sem for n = 4
*, ** significantly different from AM;
#, ## significantly different from LST;
$p < 0.05$ and $p < 0.01$, respectively

values in the morning ($p < 0.01$). In addition, the elevated levels of sucrase and lactase activities seen in the rats fed the HST diet were accompanied by elevated levels of the corresponding immunoreactive proteins ($p < 0.01$; Table 5). Columns c & f show the ratios of enzyme activity to immunoreactive protein (S/IRS and L/IRL); when these ratios were compared between night and morning within the same dietary groups, they were found to be similar with the exception of S/IRS in the HST group, which was slightly (16%), but significantly elevated at night ($p < 0.01$). This suggests that the elevated levels of enzyme activity of sucrase and lactase seen at night is primarily due to an increase in corresponding immunoreactive proteins.

In contrast, when the ratios of enzyme activity to immunoreactive protein were compared between the two dietary groups, (i.e. LST versus HST), significantly higher ratios of S/IRS and L/IRL in the HST group were seen, with one exception; L/IRL at night (Table 5). Thus, increasing the carbohydrate content of the diet appears to lead not only to an increase in enzyme activity and immunoreactive protein of these disaccharidases, but also to some increase in the enzyme catalytic efficiency.

Diurnal Variation Of The Dietary Adaptation Of Disaccharidase Activities

Based on the information obtained in the previous two experiments, further experiments were performed tc see whether the adaptation of disaccharidases to a "short term" (6 and 12 hour) increase in dietary carbohydrate depends on the time of the day - in other words, whether disaccharidase activities would be influenced differently at a time when the disaccharidase activity was decreasing (i.e. early morning hours) or increasing (i.e. early evening hours).

Since a statistically significant difference was established between disaccharidase activities at 9 - 10am and 9 - 10pm, these two time periods were again selected. Sixteen rats were fed a low starch diet ad libitum, while eight other rats were fed a moderate sucrose (40% cal) diet. After one week, eight of the low starch group were force fed a liquified form of the same low starch diet (LST/LST) for 6 hours; the remaining eight of the low starch group were force fed a liquified form of the sucrose diet (LST/SUC) for 6 hours. The rats fed the sucrose diet ad libitum remained on this same diet ad libitum (SUC/SUC), until sacrifice (Experiment 3; See Figure 3).

There was no difference in body weights or small intestinal protein content between the three dietary groups (Table 6), thus, disaccharidase activities in the proximal jejuno-ileum are shown only in terms of specific activities

Table 6. Summary of body weights and small intestinal protein content of rats on LST/LST, LST/SUC or SUC/SUC diet (Exp 3)

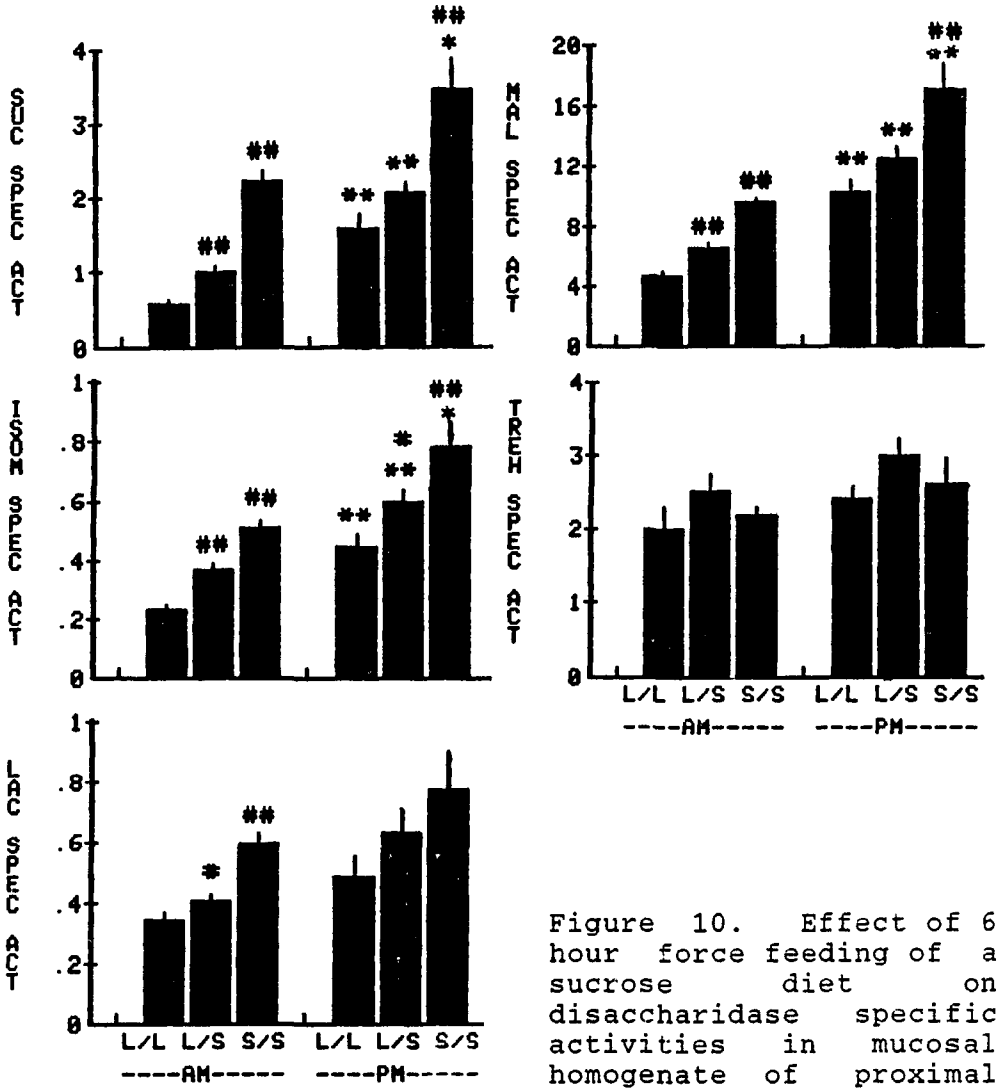
DIET	BW, DAY 0(a)	BW, DAY 6(b)	PRO/SEG(c)
LST/LST	172.5 ± 4.0	183.3 ± 3.4	143.1 ± 3.7
LST/SUC	173.0 ± 2.6	183.3 ± 2.9	143.7 ± 3.7
SUC/SUC	174.8 ± 3.4	190.2 ± 2.8	144.0 ± 7.6

(a) = body weight at beginning of experiment, grams
(b) = body weight one day before beginning force feeding, grams
(c) = protein content per small intestinal segment, mg (proximal jejuno-ileum)

see text for explanation of diets and experimental design; values represent mean ± sem for n = 8

(μmoles substrate hydrolzed per hour per mg protein). As shown in Figure 10, sucrase, isomaltase, and maltase activities of rats sacrificed at night were significantly higher than those sacrificed in the morning, regardless of dietary regime. The level of lactase activity at night was also higher than in the morning, but the difference did not reach a significant level. In contrast to the other disaccharidases, trehalase activity was similar in the morning and at night.

"Short term" (6 hour) force feeding of the sucrose diet to rats initially fed a low starch diet led to an increased level of disaccharidase activity (Figure 10). This increase was significant for sucrase, isomaltase, lactase, and maltase activities in the morning, but only for isomaltase at night. Trehalase activity tended to increase in the morning and at night, but did not reach a level of significance. The increased levels of sucrase, isomaltase, lactase and maltase activities of the rats on the LST/SUC diet did not reach, in six hours, the levels of corresponding disaccharidase activities of rats who received the sucrose diet ad libitum throughout the entire experimental period (SUC/SUC; Figure 10). In the animals kept on the sucrose diet, trehalase activity was similar to the level seen in rats fed only the low starch diet (LST/LST), whereas sucrase, isomaltase, lactase, and maltase



*,** signif diff from AM;
#,## signif diff from LST;
$p < 0.05$ & $p < 0.01$, respectively

L/L = LOW STARCH/LOW STARCH
L/S = LOW STARCH/SUCROSE
S/S = SUCROSE/SUCROSE

Figure 10. Effect of 6 hour force feeding of a sucrose diet on disaccharidase specific activities in mucosal homogenate of proximal jejuno-ileum of rats (Exp 3)

specific activity is expressed as µmoles substrate hydrolyzed per hr per mg protein; values represent mean ± sem for n=4

activities were significantly higher in the SUC/SUC group when compared to the LST/LST group (Figure 10).

When the increase in disaccharidase activities in response to "short term" feeding of the sucrose diet was expressed either in absolute units (specific activity) or as percentage of change (Table 7), an interesting phenomenon was seen. A greater percentage of change occured in the morning (e.g. sucrase specific activity increased by 70% in the morning, but only 28% at night), prompting a conclusion that the disaccharidase activity was more influenced by a change in carbohydrate content of the diet when disaccharidase activity was falling (i.e. in the morning). But when the increase in disaccharidase activities was expressed as absolute units, an identical increase was seen in the morning and at night (e.g. sucrase specific activity increased 0.41 $\pm$ 0.05 units in the morning and 0.46 $\pm$ 0.16 units at night). Thus, the magnitude of increase in disaccharidase activity in response to adding sucrose to the diet was always the same for each disaccharidase in the morning and at night, and was independent of the actual level of disaccharidase activity at the time the sucrose diet was started.

Table 7. Comparison of daytime versus nighttime response of disaccharidase specific activities to 6 hour force feeding of a sucrose diet (Exp 3)

	AS "UNITS OF DIFFERENCE" (a)		AS "PERCENTAGE OF CHANGE" (b)	
	AM	PM	AM	PM
SUCRASE	0.41 ± 0.05	0.46 ± 0.16	70.39	28.34
ISOMALTASE	0.13 ± 0.02	0.15 ± 0.04	61.50	35.07
LACTASE	0.15 ± 0.02	0.14 ± 0.06	42.73	28.34
MALTASE	1.89 ± 0.55	2.26 ± 0.80	41.34	22.34
TREHALASE	0.54 ± 0.28	0.57 ± 0.15	26.33	23.24

(a) = "units of difference" of specific activity; mean ± sem of "units of difference" was calculated by subtracting the individual specific activity value of LST/SUC group from the mean specific activity value of the LST/LST group; n = 7 - 8

(b) = values of "percentage of change" was calculated using the mean specific activity values of the LST/LST group and LST/SUC group; n = 7 - 8

The results of the electroimmuno assay are shown in Table 8. Again, to ensure valid comparisons, efficacy of papain treatment was evaluated. Recoveries of sucrase, isomaltase and lactase activities in the papain supernatant were not as high as obtained in Experiment 2 (approximately 56%, 37% and 36%, respectively, compared with disaccharidase activities in the homogenate), but were consistant between LST/LST, LST/SUC, and SUC/SUC groups, and therefore acceptable for use.

The 6 hour feeding of the sucrose diet (LST/SUC) did not affect the S/IRS and L/IRL ratios over those found in the LST/LST group in the morning or at night (Table 8, columns c and f). In contrast, S/IRS and L/IRL ratios in rats fed the sucrose diet for the entire experimental period (SUC/SUC), were significantly higher ($p < 0.01$) than ratios found in the LST/LST group which was killed in the morning hours (Table 8).

The final experiment was an extension of the 6 hour feeding experiment; in this experiment, a 12 hour force feeding was employed and the SUC/SUC regime was eliminated. Sixteen rats were fed a low starch diet ad libitum for one week, and then force fed a liquified low starch diet ($n = 8$) or a liquified sucrose diet ($n = 8$) for 12 hours (Experiment 4; See Figure 4). Four animals from each group were sacrificed at 10am and 10pm. In addition to disaccharidase

Table 8. Enzyme activity and immunoreactive amounts of sucrase and lactase in the papain supernatant of the proximal jejuno-ileum of rats on LST/LST, LST/SUC or SUC/SUC diet (Exp 3)

	SA(a)	IRS(b)	S/IRS(c)
AM:			
LST/LST	2.73 + 0.69	7.75 + 1.00	0.35 + 0.05
LST/SUC	3.88 + 0.85	8.29 + 1.08	0.45 + 0.05
SUC/SUC	8.21 + 1.27##	11.62 + 0.86#	0.70 + 0.06##
PM:			
LST/LST	7.97 + 1.21**	13.20 + 1.94*	0.62 + 0.09*
LST/SUC	11.29 + 1.54**	14.38 + 1.36*	0.78 + 0.07**
SUC/SUC	18.89 + 5.84	20.67 + 3.12*	0.86 + 0.16

	LA(d)	IRL(e)	L/IRL(f)
AM:			
LST/LST	1.01 + 0.11	3.44 + 0.25	0.29 + 0.01
LST/SUC	1.16 + 0.22	3.59 + 0.49	0.32 + 0.02
SUC/SUC	1.45 + 0.19	3.79 + 0.29	0.38 + 0.02##
PM:			
LST/LST	1.61 + 0.21*	5.02 + 0.71	0.32 + 0.03
LST/SUC	2.25 + 0.40*	5.63 + 0.75	0.39 + 0.03
SUC/SUC	2.61 + 0.83	6.45 + 1.49	0.38 + 0.03

(a) = sucrase specific activity, µmoles substrate hydrolyzed per hour per mg protein
(b) = immunoreactive sucrase, ug/mg protein
(c) = sucrase activity/immunoreactive sucrase
(d) = lactase specific activity, µmoles substrate hydrolyzed per hour per mg protein
(e) = immunoreative lactase, ug/mg protein
(f) = lactase activity/immunoreactive lactase

see text for explanation of diets and experimental design; values represent mean ± sem for n = 3 - 4
*, ** significantly different from AM;
#, ## significantly different from LST/LST;
$p < 0.05$ and $p < 0.01$, respectively

and protein assays of mucosal homogenate, sucrase and lactase activities were determined in horizontal slices of the proximal jejuno-ileum to define the locus of change in disaccharidase activity along the villus-crypt unit in response to the 12 hour increase in dietary carbohydrate content.

No difference in body weights or small intestinal protein content was seen between the dietary groups, LST/LST or LST/SUC (Table 9). Therefore, disaccharidase activities are only presented as specific activities (µmoles substrate hydrolyzed per hour per mg protein).

Feeding the sucrose diet for 12 hours to rats previously fed the low starch diet resulted in a significant increase in sucrase, isomaltase, lactase, and maltase activities both in the morning and at night (Figure 11; trehalase activity was not measured). Whereas in the 6 hour feeding experiment (Experiment 3), the increases in sucrase, lactase, and maltase activities at night did not reach a significant value, in this 12 hour feeding experiment, all four disaccharidases showed significant increases during the morning and night.

As observed with the 6 hour force feeding (Experiment 3), the magnitude of the increase in disaccharidase activities in response to the 12 hour force feeding of the sucrose diet was identical for each

Table 9. Summary of body weights and small intestinal protein content of rats on LST/LST or LST/SUC diet (Exp 4)

DIET	BW,DAY 0(a)	BW,DAY 6 (b)	PRO/SEG(c)
LST/LST	201.0 ± 6.5	208.9 ± 5.6	166.3 ± 8.8
LST/SUC	199.6 ± 9.5	205.9 ± 7.5	165.3 ± 12.2

(a) = body weight at beginning of experiment, grams
(b) = body weight on day before beginning force feeding, grams
(c) = protein content per small intestinal segment, mg (proximal jejuno-ileum)

see text for explanation of diets and experimental design; values represent mean ± sem for n = 8

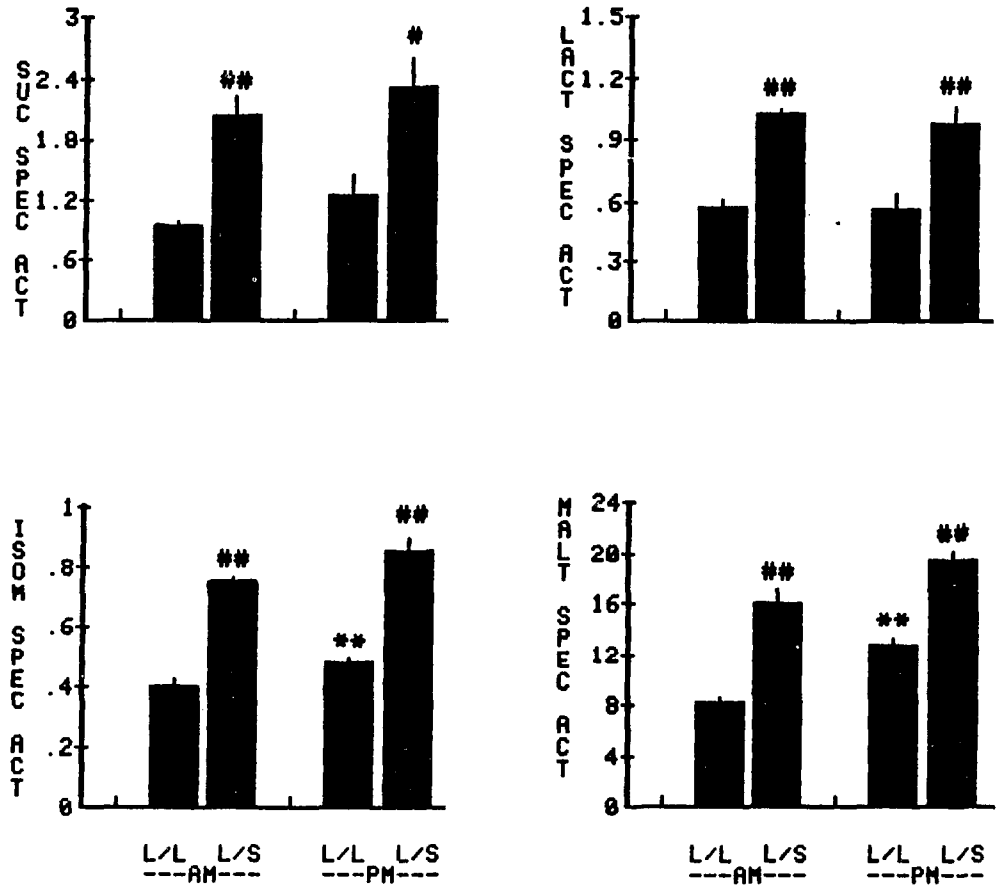


Figure 11. Effect of 12 hour force feeding of a sucrose diet on disaccharidase specific activities in mucosal homogenate of proximal jejuno-ileum of rats (Exp 4)

specific activity is expressed as μmoles substrate hydrolyzed per hr per mg protein; values represent mean ± sem for n=4;
*,** signif diff from AM; #,## signif diff from L/L; $p<0.05$ & $p<0.01$, respectively

L/L = LOW STARCH/LOW STARCH
L/S = LOW STARCH/SUCROSE

disaccharidase, regardless of the time of day when the sucrose diet was given, day or during the night (Table 10).

The changes in sucrase and lactase activities along the villus-crypt units of the proximal jejuno-ileum in response to the 12 hour force feeding of the sucrose diet are shown in Figures 12 and 13. In rats force fed the low starch diet from 10pm to 10am (i.e. LST/LST; sacrificed at 10am), the peak of sucrase activity was located at the middle villus region. Force feeding of the sucrose diet during this same time period (i.e. LST/SUC; sacrificed at 10am) led to an increase of sucrase activity at the lower to middle villus region, resulting in a slight shift of the peak towards the lower villus in these rats. In contrast, lactase activity peaked at a more apical location in the LST/LST group, and this peak remained near the middle to upper villus upon feeding of the sucrose diet for 12 hours.

In rats force fed the sucrose diet from 10am to 10pm (i.e. LST/SUC; sacrificed at 10 pm), the locus of increase in sucrase and lactase activity was essentially the same as observed in rats force fed the low starch diet (LST/LST) and sacrificed at 10am (Figures 12 and 13).

Table 10. Comparison of daytime versus nighttime response of disaccharidase specific activities to 12 hour force feeding of a sucrose diet (Exp 4)

	AS "UNITS OF DIFFERENCE" (a)		AS "PERCENTAGE OF CHANGE" (b)	
	AM	PM	AM	PM
SUCRASE	1.09 ± 0.04	1.07 ± 0.20	118.38	87.24
ISOMALTASE	0.35 ± 0.02	0.38 ± 0.01	90.45	80.63
LACTASE	0.44 ± 0.04	0.42 ± 0.07	77.66	74.77
MALTASE	7.92 ± 0.38	6.74 ± 0.54	97.88	53.18

(a) = "units of difference" of specific activity; mean ± sem of "units of difference" was calculated by subtracting the individual specific activity value of LST/SUC group from the mean specific activity value of the LST/LST group; n = 7 - 8

(b) = values of "percentage of change" was calculated using the mean specific activity values of the LST/LST and LST/SUC group; n = 7 - 8

Figure 12. Effect of 12 hour force feeding of a sucrose diet on sucrase and lactase specific activities along the villus-crypt units of proximal jejuno-ileum of rats fed from 9pm - 9am (Exp 4)

Abscissa = height of intestinal wall, with 100% representing the villus tip and 0% representing the serosa; values represent mean $\pm$ sem for n = 3-4; specific activity = μmoles substrate hydrolyzed per hour per mg protein

open circles = low starch/low starch
closed circles = low starch/sucrose

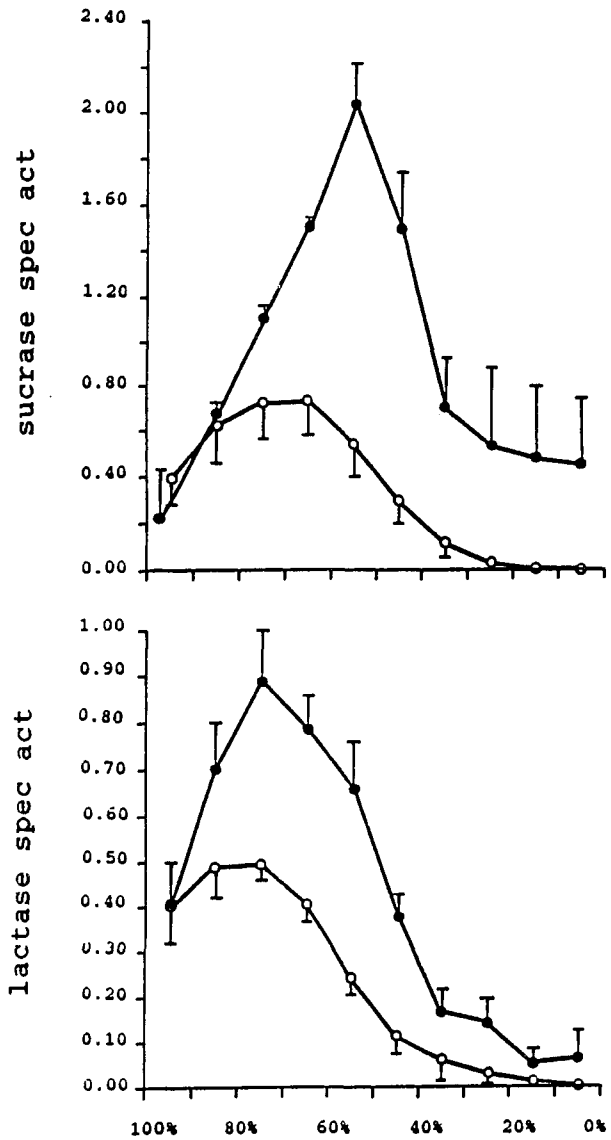


Figure 12. Effect of 12 hour force feeding of a sucrose diet on sucrase and lactase specific activities along the villus-crypt units of proximal jejuno-ileum of rats fed from 9pm - 9am (Exp 4)

Figure 13. Effect of 12 hour force feeding of a sucrose diet on sucrase and lactase specific activities along the villus-crypt units of proximal jejuno-ileum of rats fed from 9am - 9pm (Exp 4)

Abscissa = height of intestinal wall, with 100% representing the villus tip and 0% representing the serosa; values represent mean $\pm$ sem for n = 3-4; specific activity = μmoles substrate hydrolyzed per hour per mg protein

open circles = low starch/low starch
closed circles = low starch/sucrose

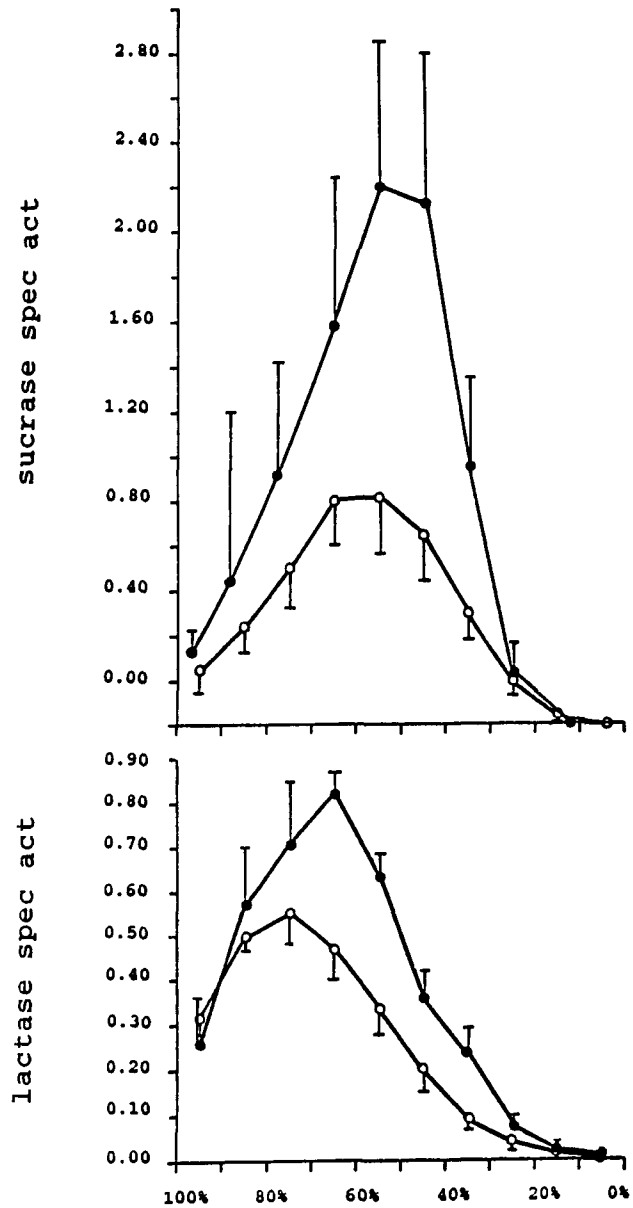


Figure 13. Effect of 12 hour force feeding of a sucrose diet on sucrase and lactase specific activities along the villus-crypt units of proximal jejuno-ileum of rats fed from 9am - 9pm (Exp 4)

DISCUSSION

The primary objective of this thesis was to look at the "short term" (6 and 12 hour) response of disaccharidase activities to feeding of a high carbohydrate diet and to evaluate how the time of day (i.e. AM, when disaccharidase activities are falling or PM, when disaccharidase activities are rising) influences this response. To assess the "short term" response, the diurnal rhythm, and the "long term" (1 week or longer) response of disaccharidase activities to a high carbohydrate diet had to be addressed first. The conclusions reached are presented in three sections: (a) diurnal rhythm of disaccharidase activities; (b) adaptation of disaccharidase activities to "long term" feeding of a high starch diet (i.e. when a new steady state is established); (c) adaptation of disaccharidase activities to "short term" feeding of a sucrose containing diet (i.e. before a new steady state is established).

Diurnal Rhythm of Disaccharidase Activities

The presence of a diurnal rhythm of sucrase, isomaltase, lactase, and maltase activities in the proximal jejuno-ileum of rats was demonstrated in Experiment 1 and confirmed in Experiment 2. In agreement with others (Saito et al., 1975; Saito et al., 1976a; Saito et al., 1976b; Nishida et al., 1978; George et al., 1985), a broad peak in disaccharidase activities was seen between 9pm and 3am, and a nadir was seen between 9am and 12pm (Figures 6, 7, and 9).

This diurnal pattern of sucrase, isomaltase, lactase and maltase activities was present in rats fed a high starch diet, as well as in those fed a low starch diet. These results are in accordance with the results of Saito et al. (1978) which demonstrated that a circadian rhythm of sucrase and maltase activities exists even when rats are fed a carbohydrate free diet. In this study, a diurnal pattern of trehalase activity was also seen, but only in rats fed the high starch diet. This exception is interesting, but there is no good explanation for this at present. It may be related to the known dissimilarity of trehalase and the four other disaccharidases. First, although trehalase is not susceptible to pancreatic proteases (as are other disaccharidases), trehalase is susceptible to lysosomal proteases (Seetharam et al., 1976). Second, trehalase is

thought to be localized more deeply in the brush border membrane than the other disaccharidases, and therefore less likely to contact dietary contents.

The pattern of rise and fall of sucrase, isomaltase, lactase, and maltase activities was similar to the pattern of rise and fall of food intake, when diet was fed ad libitum, as reported by Bolles (1965), Stevenson et al. (1976), and Saito et al. (1976a, 1976b). Siegel (1961) reports a maximum intake of food between 7pm and 10pm, a trough between 10pm and 2am, a second maximum between 2am and 5am, and a minimum of food intake between 7am and 12 noon. This general pattern of food intake was confirmed in this study (Table 5), with a maximum seen between 6pm and 9pm, a trough between 9pm and 3am, a second maximum between 3am and 6am, and a minimum of intake from 6am to 9am. It should be pointed out that both dietary groups showed the same diurnal food intake pattern.

The rise of disaccharidase activities is reported to slightly precede the beginning of food intake (Saito et al., 1976a; Saito et al., 1976b). But even when rats are starved, the circadian rhythm of sucrase and maltase activities persist for several days, then eventually disappear (Saito et al., 1976b). Thus, the actual food intake does not seem to be the sole stimulus for the rise and fall of disaccharidase activities. Rather, the

"anticipatory effect" of a routine food schedule may be the important element in predicting the diurnal pattern of disaccharidase activities (Saito et al., 1976a; Saito et al., 1976b; George et al., 1985).

It has been reported that the degradation of sucrase exhibits a diurnal variation which might be responsible for the diurnal variation of sucrase activity (Kaufman et al., 1980). To investigate whether the diurnal fluctuation in disaccharidase activities is related to a change in the activity of luminal proteases, luminal trypsin activity was evaluated at 9am, 12pm, 3pm, 9pm, 12am, and 3am (Figure 8). Trypsin is the activated form of trypsinogen and is necessary for the activation of other proteolytic enzymes, so the amount of (already) active trypsin in the luminal flush was used as an indicator of representative luminal proteolytic activity. No diurnal pattern of luminal trypsin activity, which could account for the diurnal fluctuation of sucrase, isomaltase, lactase or maltase activities was seen. These results are in accordance with the results of Girand-Glober et al. (1980) and George et al. (1985). Since chymotrypsinogen content in the pancreas has been reported to exhibit a diurnal pattern (Girand-Glober et al., 1980), chymotrypsin activity in the luminal contents should be

evaluated in a future study and compared to the diurnal variation in disaccharidase activities.

A comparison of the ratios of sucrase activity to immunoreactive sucrase, and lactase activity to immunoreactive lactase (S/IRS and L/IRL, respectively), indicated that the diurnal increase in enzyme activity that occurs at night was due to a parallel increase in enzyme activity and imunoreactive protein, rather than an increase in catalytic efficiency of sucrase or lactase (Experiment 2; Table 5). This agrees with the report on sucrase activity by Kaufman et al. (1980).

Some conclusions can be drawn from these preliminary experiments: (1) there was a close relationship between the diurnal fluctuations in disaccharidase activities and food intake patterns; (2) a diurnal fluctuation of disaccharidase activitiy was maintained in rats fed both the low starch and the high starch diets; (3) luminal trypsin activity did not appear to control the diurnal fluctuation of disaccharidase activities; and (4) the diurnal fluctuations of both sucrase activity, an α-glucosidase, and lactase activity, a β-galactosidase were accompanied by a parallel change in the corresponding immunoreactive protein (i.e. an actual change in amount of enzyme was seen). These last three conclusions are very important when both the "long term" and

the "short term" response of disaccharidase activities to dietary carbohydrate are examined, and will be refined and discussed further in the following sections.

Adaptation Of Disaccharidase Activities To "Long Term" Feeding Of A High Starch Diet

In agreement with previous investigations (Bustamante et al., 1981; Yamada et al., 1981b; Riby and Kretchmer, 1984; Leichter et al., 1984), sucrase, isomaltase, lactase, and maltase activities were significantly ($p < 0.01$) higher in the HST group than the LST group, when rats were fed these diets for one week (Experiment 2; Figure 9). Trehalase activity, again, was the exception (trehalase activity was significantly higher in the HST group, but only at night). Thus, trehalase appears to respond differently to carbohydrate intake than the other four disaccharidases - the diurnal fluctuation in trehalase activity was not apparent when the carbohydrate content of the diet was low (i.e. LST diet; Exp 1). When trehalase activity was compared in the LST and HST groups, it was significantly increased only at night. It seems that a certain "threshold" of carbohydrate intake, either from an increase in the porportion of carbohydrate in the diet, or from an increase in the quantity of carbohydrate consumed, is required to provoke a significant response in trehalase activity.

Trypsin activity in the lumen of the proximal, middle, and distal segments of the jejuno-ileum was similar in both the LST and HST groups (Table 4). However, this was expected, because the protein content of both diets were kept equal (Table 1). These results suggest that the effect of carbohydrate intake on disaccharidase activities is probably not due to the modification of pancreatic secretions, or at least not by a modification of trypsin activity. However, the following possibilities should not be excluded; some other pancreatic protease (e.g. elastase, chymotrypsinogen) may be selectively secreted or activated in rats fed the LST diet, and may be responsible for the increased degradation and resultant lower disaccharidase levels seen in the LST group. Other non-protease factors, such as luminal pH or bile acids, might also affect the degradation of disaccharidases. These possibilities should be examined further.

Riby and Kretchmer (1984) recently demonstrated that the rate of incorporation of a precursor amino acid, <3H>-leucine, into sucrase was significantly (2.6 times) higher in rats fed a high carbohydrate (sucrose) diet than those fed a carbohydrate free diet, suggesting that the rate of synthesis of sucrase-isomaltase is altered by the amount of dietary carbohydrate. Tsuboi et al. (1985) also reported increases in sucrase and maltase activities as a result of

de novo synthesis of the enzyme proteins, when rats were switched from a low carbohydrate to a high carbohydrate diet. However, it is not clear whether the rate of synthesis of other disaccharidases are also modified by the change in carbohydrate intake. Since all disaccharidases are glycoproteins, it's possible that an increase in carbohydrate intake provides more "building blocks" to be used in increased enzyme synthesis.

The same authors also estimated the degradation rate of sucrase and found it to be 20% slower in rats fed a high carbohydrate diet. This might indicate that sucrase, and conceivably other disaccharidases, are stabilized or protected by a high carbohydrate diet. Also, an excess of carbohydrate in the lumen may provide a barrier to guard enzymes from degradative activity, either by direct contact with the enzyme catalytic site or by stearic hinderance. In this context, it should be mentioned that an increased catalytic efficiency of both sucrase and lactase activities (estimated by S/IRS and L/IRL ratios), as a result of an increased carbohydrate content of the diet, was seen (Exp 2; Table 5). Both S/IRS and L/IRL ratios were increased in the morning, as was S/IRS at night, when the LST versus HST groups are compared. There is no explanation for the lack of

similar trend in L/IRL at night, except that lactase seems to be more resistant to change by dietary carbohydrate than sucrase.

The increase in S/IRS and L/IRL ratios seen in the rats fed the HST diet might indicate that the inactivation of the catalytic site of sucrase and lactase (as part of the degradation process) was retarded. Alternatively, an enzyme form with a different property (i.e. different carbohydrate moiety or different catalytic efficiency) might be synthesized.

Another possibility is that a low ratio of enzyme to pancreatic protease (as would be found in the LST diet group) would increase the enzymes' susceptibility to degradation by proteases. This idea was suggested by Seetharan et al. (1980), who reported that lactase activity in lactase deficient individuals to be more sensitive to pancreatic proteases. But the susceptibility of enzymes to degradation is a characteristic of the enzymes themselves, and it is not likely that sensitivity to degradation is dependent on the concentration of the enzymes.

The conclusion that can be drawn from this "long term" disaccharidase response experiment is that the levels of both α-disaccharidases and β-disaccharidases are dependent on the carbohydrate (starch) intake. The increased levels of disaccharidase activities seen in rats

fed the high starch diet was observed not only in the morning (at the time of nadir in enzyme activities) but also at night (at the time of peak in enzyme activities). This experiment also showed that the regulation of disaccharidase activities due to diurnal factors is different from the regulation of disaccharidase activities due to dietary carbohydrate; whereas the diurnal fluctuation in disaccharidase activities is a result of parallel increase in enzyme activity and immunoreactive protein, the "long term" dietary adaptation in disaccharidase activity also involved some (in)activation of the enzyme.

Adaptation Of Disaccharidase Activities To 6 and 12 Hour Feeding Of A Sucrose Diet

The "short term" response of disaccharidase activities to an increase in dietary carbohydrate in the morning and at night was evaluated using a "force feeding" technique, in which calculated amounts of diet were fed by gavage to each rat so that the intake between rats could be regulated. Controlling the food intake between dietary groups was important because (1) lactase is known to respond differently than the other disaccharidases to starvation or a reduced intake of food (Yamada et al., 1983); (2) ad libitum food intake varies at different times of the day

(Siegel, 1961); and (3) there is considerable variation in the timing of food intake among individual rats when fed ad libitum.

This force feeding procedure has previously been performed successfully (Goda et al., 1984a; Goda et al., 1985d). In both of these studies, no significant difference in body weights, food intake, or total jejunal mucosa protein is seen among rats force fed a low starch diet, a 40% sucrose diet, a 40% lactose diet, or rats fed a low starch diet ad libitum. In the 1985 study, the height of the intestinal wall, height of the villus, and the rate of cell migration (<3H>-thymidine injected at the same time force feeding began) in the proximal jejuno-ileum of these rats were also evaluated and were not found to be significantly different from each other. These two studies report no change in S/IRS and L/IRL ratios following 3 - 12 hours of the force feeding. It should be noted that in these studies, the rats were always sacrificed in the morning (10am).

In experiments 3 and 4, no significant difference in body weight gains or small intestinal protein content was seen among the dietary groups (Tables 6 and 9), substantiating the validity of the experimental procedure.

When the rats were force fed the sucrose diet for 6 and 12 hours, a dietary-induced increase in sucrase, isomaltase, lactase and maltase activities was seen (Figures 10 and 11). A significant increase in sucrase, isomaltase, lactase and maltase activities was observed as early as 6 hours after the initial force feeding of the sucrose containing diet in the rats sacrificed in the morning. Force feeding of the sucrose containing diet for 12 hours led to a significant increase of these disaccharidase activities at both time periods. It should be noted that the rats were force fed the same amount of diet at both time periods. The lack of statistical significance for the rats force fed for 6 hours and sacrificed at night could be explained by the larger scatter of values in disaccharidase activities of those rats.

When the increase in disaccharidase activities following force feeding of the sucrose diet was closely examined, a very interesting phenomenon was seen. Feeding of the sucrose diet to rats which had been fed the low starch diet caused essentially identical increases in sucrase, isomaltase, lactase, maltase, and trehalase specific activities in the morning and at night. This implies that the dietary adaptation of disaccharidases is independent of the diurnal variation of disaccharidase activities (i.e. independent of whether disaccharidase activities are rising

or falling at the time the new diet is introduced) and that it is independent of the initial level of disaccharidase activity.

In these "short term" force feeding experiments, the amount of diet force fed was chosen to provide quantitatively "normal" amount of food delivery to rats sacrificed in the morning, and an equivalent amount was given to the rats sacrificed at night. The identical increases in disaccharidase activities seen at both time periods reinforces the importance of the amount of dietary carbohydrate in influencing disaccharidase levels, since an identical increase was seen in both groups although disaccharidase activities were falling in the group sacrificed in the morning and rising in the group sacrificed at night. Furthermore, if the data in Tables 7 and 10, are compared, one sees that the increase in disaccharidase activities as a result of the 12 hour feeding of the sucrose diet was approximately three times that seen in the 6 hour feeding of the same diet. Since in the 12 hour force feeding experiment, rats were provided an amount of diet (20 kcal/100 g body weight) which was approximately triple that fed to rats in the 6 hour force feeding experiment (7.5 kcal/100 g body weight), it appears that the change in

disaccharidase activity is a direct consequence of the amount of dietary carbohydrate ingested.

A comparison of ratios of enzyme activity to immunoreactivity between the LST/LST and LST/SUC groups showed only a trend of increasing enzyme activity to immunoreactivity in the rats force fed the sucrose diet; no significant difference was observed (Experiment 3; Table 8). In the previous "long term" feeding experiment (Experiment 2; Table 5), the dietary adaptation of sucrase and lactase activities to the high starch diet resulted in not only an increase in enzyme amount but also some enzyme activation (or decrease in inactivation). Presumably, this difference was due to the lower carbohydrate content of the sucrose diet (70% starch versus 40% sucrose) and the shorter length of time on the high carbohydrate diets (1 week versus 6 hours).

To determine the locus of enterocytes along the villus-crypt unit which exhibits the disaccharidase adaptation to dietary sucrose, a portion of the proximal jejuno-ileum was sliced horizontally using the technique of cryostat sectioning. Sucrase activity is normally expressed maximally in the mid villus region (approximately 45 - 65% from the bottom of the crypt), and lactase activity in the mid to upper villus region (approx. 65 - 85% from the bottom of the crypt) (Boyle et al., 1980). Figures 12 and 13

reveal that the maximal increase in sucrase activity (in response to the 12 hour feeding of the sucrose diet) was seen in the lower to middle villus region in rats sacrificed at both time periods. In contrast, lactase activity was increased at a more apical and broader locus of enterocytes, and the distribution of lactase activity was maintained along the villus-crypt unit. Since 12 hours is less than the time necessary for a new generation of immature enterocytes to migrate to the middle villus region, this confirms that the mature enterocytes, as well as immature enterocytes, reacted to the change in dietary carbohydrate (with an increase in sucrase and lactase activities).

These same conclusions were reached by Goda et al., (1985d), but that report only looked at disaccharidase activities in rats fed a sucrose containing diet at a time when disaccharidase activities are decreasing (i.e. sacrificed in the morning). The present study expands these results by demonstrating that the site of maximal stumulation of sucrase and lactase activities is constant regardless of the time of day the dietary stimulation is given.

The major conclusion drawn from these "short term" feeding experiments was that disaccharidase adaptation to an increase in dietary carbohydrate was the same in terms of

magnitude and the locus of maximal stimulation along the villus-crypt unit, whether the new diet was introduced while disaccharidase activities were rising or falling. Furthermore, the adaptation was quite quick (expressed in less than 12 hours), and occurred in both mature and immature enterocytes of the villus-crypt unit.

The possible effect of bile acids was not addressed in these studies but might be important in the expression of disaccharidase activities (Bohmer, 1979). Dietary fat stimulates bile and bile acid release, thus, measuring the amount of bile acids in the luminal contents of both LST and HST groups could provide information as to whether or not bile is a factor in the expression of disaccharidase activities. Likewise, the role of luminal pH should also be considered, because the presence of metabolizable sugars in the lumen is accompanied by a decrease in pH on the jejunal villus surface (Daniel and Rehner, 1986).

CONCLUSIONS

A diurnal rhythm of sucrase, isomaltase, lactase, and maltase activities was present, regardless of dietary regime, i.e. low starch or high starch. A diurnal rhythm of trehalase activity was also apparent, but was maintained only in rats fed a high starch diet.

In rats switched from a low starch to an isocaloric moderate sucrose diet for 6 or 12 hours, an increase in disaccharidase activities was seen which was independent of whether disaccharidase activities were rising or falling when the sucrose diet was introduced. The absolute amount of increase in each disaccharidase (sucrase, isomaltase, lactase, maltase, and trehalase) activity and also the locus of change of sucrase and lactase activities along the villus-crypt units, were similar, regardless of the time of day the dietary carbohydrate content was altered.

These results suggest that the mechanism of the dietary adaptation of rat small intestinal disaccharidase activities is independent of the diurnal fluctuation of disaccharidase activities, and is distinct from the mechanism of the diurnal fluctuation of these enzyme activities.

APPENDIX A: COMPOSITION OF WAYNE LAB BLOX

CRUDE PROTEIN.....(minimum)....24.0%
CRUDE FAT.....(minimum).....4.0%
CRUDE FIBER.....(maximum).....4.5%

INGREDIENTS:

Corn and wheat flakes, ground corn, soybean meal, fish meal, wheat middlings, wheat red dog, dried whey, brewers dried yeast, soybean oil, animal liver meal, cane molasses, vitamin A supplement, D-activated animal sterol (source of vitamin D3), vitamin B12 supplement, vitamin E supplement, menadione sodium bisulfite complex (source of vitamin K activity), riboflavin supplement, niacin supplement, calcium pantothenate, choline chloride, folic acid, thiamine, ground limestone, calcium phosphate, salt, manganous oxide, copper oxide, iron carbonate, ethylenediamine dihydroiodine, cobalt carbonate and zinc oxide.

SOURCE: Wayne Pet Food Division, Continental Grain Company, Chicago, IL

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