Dietary-induced increases of disaccharidase activities in rat jejunum

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A study was carried out to examine whether the responsiveness of small intestinal epithelial cells to dietary carbohydrate varied during the daily 24 h cycle. The effect of sucrose on disaccharidase activities was compared during a period of decreasing disaccharidase activities, i.e. between 22.00 and 10.00 hours, and increasing disaccharidase activities, i.e. between 10.00 and 22.00 hours, in the jejunum of 7-week-old-rats. Rats were fed on a low-starch, high-fat diet (Lst; starch 5 and fat 73% of gross energy), or a high-starch, low-fat diet (Hst; starch 70 and fat 7% of gross energy). Both dietary groups exhibited typical diurnal variations in jejunal sucrase (*EC* 3.2.1.48), maltase (*EC* 3.2.1.20) and lactase (*EC* 3.2.1.23) activities, exhibiting a peak around 22.00 hours and a trough at approximately 10.00 hours. When rats were fed on diet Lst for 7 d and then force-fed on an isoenergetic sucrose diet (S; sucrose 40 and fat 37% of gross energy) for 6 or 12 h they exhibited increased sucrase, maltase and lactase activities compared with rats fed on diet Lst. The absolute increase in disaccharidase activities was similar regardless of the time diet S was given or whether rats were killed at 10.00 hours or at 22.00 hours. Analyses of sucrase and lactase activities along the villus–crypt columns showed that the distribution of cell cohorts that responded to diet S was not influenced by the time of introduction of diet S. These findings suggest that small intestinal epithelial cells possess the ability to respond to dietary carbohydrate throughout the daily 24 h cycle.

Dietary carbohydrate: Diurnal rhythm: Disaccharidase activity

Four distinct disaccharidase complexes, sucrase–isomaltase (*EC* 3.2.1.48, *EC* 3.2.1.10), glucoamylase–maltase (*EC* 3.2.1.3, *EC* 3.2.1.20), lactase (*EC* 3.2.1.23, *EC* 3.2.1.62), and trehalase (*EC* 3.2.1.28) exist in the small intestinal epithelial cells of nearly all mammals (Semenza, 1986). In the rat small intestine, a rapid alteration of disaccharidase activity can be induced by dietary manipulation. Within 3 h of feeding a sucrose-containing diet (for review, see Goda & Koldovský, 1988) the activity and immunological amount of sucrase–isomaltase (Cézard *et al.* 1983; Goda *et al.* 1985) and lactase (Goda *et al.* 1985) increase in the rat small intestine. This dietary-induced increase of sucrase and lactase activities is observed along the entire height of the villus, suggesting that not only immature crypt cells but also differentiated villus cells possess the capability of responding to a change in dietary carbohydrate (Yamada *et al.* 1981*a*; Cézard *et al.* 1983; Goda *et al.* 1985).

In addition to the dietary-induced fluctuation in disaccharidase activities, disaccharidase activities show a typical diurnal pattern. In the rat small intestine, disaccharidase activities

Table 1. *Composition of diets**

Ingredient	Low-starch		High-starch		Sucrose	
	MJ/kg	g/kg	MJ/kg	g/kg	MJ/kg	g/kg
Casein	2·66	159·0	2·63	157·0	2·78	166·0
Maize starch	0·60	36·0	8·80	526·0	—	—
Sucrose	—	—	—	—	5·15	308·0
Maize oil	9·30	247·0	0·90	24·0	4·71	125·0
AIN[76] Mineral mix†	0·06	28·0	0·06	28·0	0·06	28·0
AIN[76] Vitamin mix†	0·13	8·0	0·13	8·0	0·13	8·0
DL-methionine	0·04	2·4	0·04	2·4	0·04	2·4
Choline bitartrate	0·00	1·6	0·00	1·6	0·00	1·6
Agar (20 g/kg)	0·00	518·0	0·00	253·0	0·00	361·0
Total	12·79	1000	12·56	1000	12·87	1000

* For force-fed diets, agar was omitted and diets were diluted with water (300 ml/kg diet). Gross energy contents of diets were estimated with an assumption that carbohydrate and protein provide 16·7 kJ/g and fat provides 37·7 kJ/g. These were expressed on a dry weight basis.

† AIN[76] Mineral mix contains 118 g sucrose/kg and AIN[76] Vitamin mix contains 993 g sucrose/kg (American Institute of Nutrition, 1977).

exhibit a diurnal variation with a peak during the dark hours and a trough during the light hours (Saito, 1972; Saito *et al.* 1975; Stevenson *et al.* 1975). Food consumption appears to be an important factor affecting the diurnal variation of disaccharidase activities, since a shift in meal-time during the light hours leads to a corresponding shift in the time of peak disaccharidase activity (Saito, 1972; Saito *et al.* 1975, 1976; Stevenson *et al.* 1975). These earlier studies led us to question whether the ability of small intestinal epithelial cells to respond to dietary carbohydrate is influenced by the diurnal variation of disaccharidase activities.

MATERIALS AND METHODS

Animals

Female Sprague–Dawley rats born in the Department's animal colony were weaned at 30 d of age and fed on a standard laboratory diet (Lab Blox; Allied Mills, Chicago, IL) until 50 d of age. The rats were housed under a light cycle with 12 h darkness from 18.00 to 06.00 hours. Each treatment group consisted of four rats unless otherwise stated.

Experimental methods

Expts 1 and 2. To determine the normal diurnal variation of disaccharidase activities under different dietary carbohydrate intakes, 50-d-old rats (fourteen rats per group) were fed on either a synthetic low-starch, high-fat diet (Lst; starch 5 and fat 73% of gross energy) or a synthetic high-starch, low-fat diet (Hst; starch 70 and fat 7% of gross energy) for 14 d (Yamada *et al.* 1981*b*; see Table 1). On days 7 and 11, the rats were housed in individual cages and the food intakes measured individually every 3 h for 24 h. On day 14, the rats were killed by decapitation at 09.00, 12.00, 15.00, 21.00, 24.00 or 03.00 hours. In the second experiment, the rats were killed by decapitation at 10.00 or 22.00 hours on day 7 of the study.

Expts 3 and 4. The third and fourth studies were designed to study the short-term effects of increasing the quantity of dietary carbohydrate at different times of the day. At 50 d of

age, rats were fed on either diet Lst or sucrose diet (S; sucrose 40 and fat 37% of gross energy) for 7 d (see Table 1). On day 7, rats that were on diet Lst were switched to liquefied diets equivalent to either diet Lst or diet S. These diets were given orogastrically for 6 h (Expt 3) or 12 h (Expt 4) before the rats were killed to control precisely the dietary intakes of the two groups (Goda *et al.* 1984, 1985). Rats fed on diet S for 7 d were continued on the same diet *ad lib.* until they were killed.

The gross energy content of the liquefied diets was 9·9 kJ/ml. Force-feeding was performed three times in 12 h (Expt 3), i.e. at 22.00, 02.00 and 06.00 hours, or at 10.00, 14.00 and 18.00 hours, or twice in 6 h (Expt 4), i.e. at 04.00 and 07.00 hours, or at 16.00 and 19.00 hours. The amount of diet given was 314 kJ/kg body-weight (BW) per 6 h and 837 kJ/kg BW per 12 h. These amounts corresponded to the *ad lib.* intake of diet Lst recorded between 03.00 and 09.00 hours (for Expt 3) and between 21.00 and 09.00 hours (for Expt 4) respectively (see Fig. 1). In both experiments, the animals were killed by decapitation at 10.00 or 22.00 hours.

All synthetic diets described previously, including liquefied formulas, were prepared in this laboratory (Goda *et al.* 1984, 1985). Rats had free access to water throughout the entire experiment.

Preparation of intestinal samples

The entire small intestine was removed and the duodenum discarded, and the jejuno-ileum was divided into three segments of equal length. The proximal (upper jejunum), middle (lower jejunum) and distal (ileum) portions were separately flushed with 10 ml ice-cold saline (9 g sodium chloride/l) solution. The intestinal flushings were collected to assay for trypsin activity. The mucosa of the proximal jejunum was scraped with a glass slide on ice and homogenized with 10 mM-potassium phosphate buffer (pH 7·0). Tissue preparations and intestinal flushings were stored at $-20°$ before assaying.

Determination of disaccharidase activity gradient along the villus–crypt axis

To assay the activities of sucrase and lactase at different heights along the villus–crypt columns, a 5×5 mm segment of the middle portion of the upper jejunum was sectioned with a cryostat at $-18°$, and the gradient of these enzyme activities along the villus–crypt axis was determined as described previously (Goda *et al.* 1983).

Enzyme assays

Sucrase, lactase, maltase and trehalase activities were assayed according to the method of Dahlqvist (1964). The lactase assay mixture contained *p*-hydroxymercuribenzoate (Aldrich, Milwaukee, WI) to inhibit any residual lysosomal acid β-galactosidase ($EC\ 3.2.1.23$) activity (Koldovský *et al.* 1969). Trypsin activity was determined according to the method of Erlanger *et al.* (1961) without preincubation with enterokinase ($EC\ 3.4.21.9$). All enzyme determinations were made under conditions of linear activity with time and concentration of the enzyme. Protein was determined according to the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Statistical analysis

Results were expressed as means with their standard errors. Means of the different groups were compared by one-way analysis of variance, followed by Student's *t* test. When the effects of dietary manipulations and the effects of time were analysed simultaneously, a two-way analysis of variance was performed. For statistical analysis on the repeated

measurements over time and over the length of the villus–crypt columns, a separate analysis of variance was carried out for each height of the villus–crypt columns.

RESULTS

Expts 1 and 2. Diurnal variation of disaccharidase activities in rats fed on the low- and high-starch diets

To examine whether the level of dietary carbohydrate affects the diurnal variation of small intestinal disaccharidase activities, rats were fed on either diet Lst or diet Hst *ad lib.* for 14 d. Food intake for a 24 h period on day 7 was recorded at 3 h intervals to compare the pattern of *ad lib.* intake between the two dietary groups. Except for a 3 h period between 21.00 and 24.00 hours, rats of both dietary groups consumed comparable amounts throughout the 24 h period (Fig. 1). Between 21.00 and 24.00 hours, the rats fed on diet Lst consumed 28 % less than the rats fed on diet Hst ($P < 0.05$). However, there was no significant difference in total energy intake per kg BW over the 24 h period. Mean daily energy intake on diet Lst was 1460 (SEM 75) kJ/kg BW (n 14) and on diet Hst 1670 (SEM 92), kJ/kg BW (n 14). Being nocturnal, rats in both dietary groups consumed more energy ($P < 0.001$) during the dark hours of 18.00–06.00 hours when mean energy intake on diet Lst was 970 (SEM 59), kJ/kg BW (n 14) and on diet Hst was 1140 (SEM 38) kJ/kg BW (n 14), than during the light hours of 06.00–18.00 hours when mean energy intake on diet Lst was 490 (SEM 50) kJ/kg BW (n 14) and on diet Hst 540 (SEM 80) kJ/kg BW (n 14; see Fig. 1). The diurnal pattern and the absolute amount of *ad lib.* intake of the two dietary groups on day 11 was similar to that observed on day 7 (values not shown).

After giving diets Lst or Hst for 7 d, no significant difference in BW, jejunal mucosal weight or total jejunal mucosal protein was observed between the two dietary groups (values not shown). Furthermore, the jejunal mucosal weight and the jejunal total protein in rats killed at 10.00 hours was similar to that of rats killed at 22.00 hours regardless of diet consumed (values not shown).

In Expt. 1 we saw a cyclic diurnal pattern of increasing and decreasing sucrase, maltase and lactase activities in the jejunum of rats fed on diets Lst and Hst *ad lib.* for 14 d (Fig. 2*a–c*). The peak occurred at night (21.00–24.00 hours) and a minimum occurred approximately 12 h later (09.00–12.00 hours). A similar cyclic pattern of trehalase activity was seen in rats fed on diet Hst, but not in rats fed on diet Lst (Fig. 2*d*). Accordingly, two times, 10.00 and 22.00 hours, were selected to represent the trough and peak of disaccharidase activities in subsequent studies.

The result of the second experiment is shown in Table 2. Sucrase, maltase, and lactase activities were significantly elevated ($P < 0.001$) at 22.00 hours when compared with the corresponding activities at 10.00 hours in rats fed on diets Lst or Hst. Trehalase activity was elevated at 22.00 hours, compared with 10.00 hours, but only in rats fed on diet Hst. Sucrase, maltase and lactase activities in rats fed on diet Hst were always higher than those of rats fed on diet Lst, whether they were killed at 10.00 or 22.00 hours. In contrast, trehalase activity of rats fed on diet Hst was higher than that of rats fed on diet Lst only at night; no dietary effect was observed in rats killed at 10.00 hours (Table 2).

Thus, a diurnal variation of sucrase, maltase, and lactase activities was maintained regardless of dietary regimen, whereas a diurnal variation of trehalase activity was maintained only in rats fed on diet Hst.

Since proteases present in the lumen of the small intestine can influence disaccharidase activities (Alpers & Tedesco, 1975; Goda & Koldovský, 1985; Riby & Kretchmer, 1985; Goda *et al.* 1988), lumen trypsin activity of the proximal, middle and distal segments of the
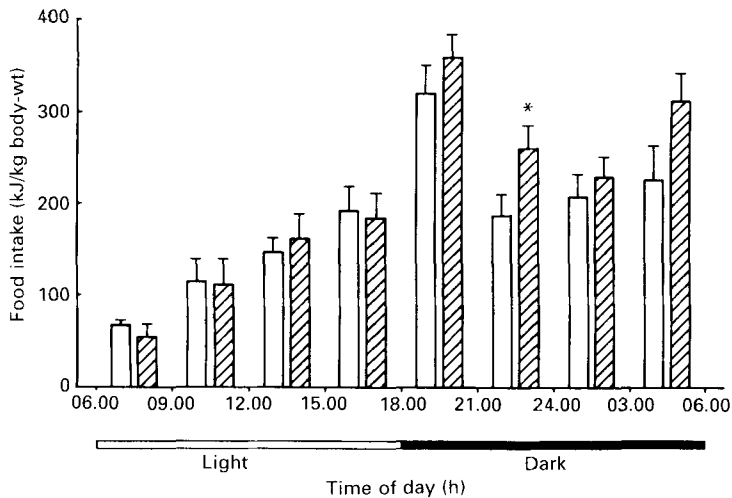
Fig. 1. Expt. 1. Energy intake (kJ/kg body-weight) during one 24 h time-period (7th day) in rats on *ad lib.* low starch (Lst; □) or high-starch (▨) diets (for details of compositions, see Table 1). Energy intakes during the 3 h time-periods were measured at 09.00, 12.00, 15.00, 18.00, 21.00, 24.00, 03.00 and 06.00 hours. Values are means of fourteen rats. Mean value was significantly different from that of rats fed on diet Lst: * $P < 0.05$.

jejuno-ileum was compared between the two dietary groups as well as between the two times. There was no diurnal variation of lumen trypsin activity in rats fed on diets Lst or Hst, nor was any difference seen in rats killed at either 10.00 or 22.00 hours (values not shown).

Expts 3 and 4. Diurnal variation of dietary-induced increase of disaccharidase activities
Based on information obtained in the first two experiments, experiments were conducted to determine if the dietary-induced modification of disaccharidase activities (i.e. response of disaccharidase activities to changes in amount of carbohydrate intake) was affected by the time of day when the diet was introduced. The aim was to establish if the response of disaccharidase activities to a change in carbohydrate intake differs if the change in carbohydrate intake occurs between 22.00 and 10.00 hours (a period of decreasing disaccharidase activities) or between 10.00 and 22.00 hours (a period of increasing disaccharidase activity).

Rats were fed on diet Lst for 7 d, then given diet S or diet Lst for 6 or 12 h. The total jejunal protein content was not significantly different between the rats fed on diet S or diet Lst for 6 or 12 h regardless of time of killing (values not shown). Force-feeding of diet S to rats initially fed on diet Lst led to a significant increase in sucrase, maltase, and lactase activities within 6 h compared with those levels in rats force-fed with diet Lst for the same time-period (Table 3). The absolute difference between the two groups in sucrase, maltase, and lactase activities at 10.00 hours was similar to that observed at 22.00 hours. However, when the difference was expressed as a percentage of the values measured in the Lst-fed group, a greater increase in sucrase, maltase, and lactase activities occurred at 10.00 hours than at 22.00 hours, i.e. sucrase activity increased by 70 % at 10.00 hours but only 28 % at 22.00 hours, maltase activity increased by 41 % at 10.00 hours but only 22 % at 22.00 hours, and lactase activity increased by 43 % at 10.00 hours but only 28 % at 22.00 hours. The elevated levels of sucrase, maltase, and lactase activities caused by force-feeding with diet S did not approach the levels of disaccharidase activities seen in rats that were fed on diet S *ad lib.* throughout the entire experimental period (results not shown). Force-feeding with
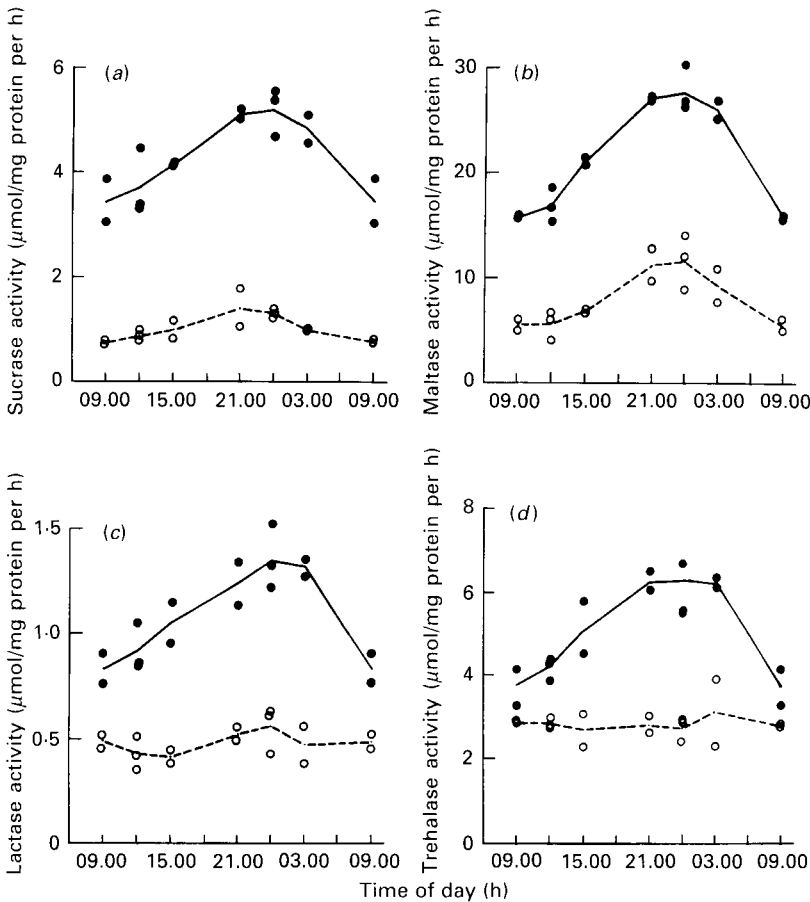
Fig. 2. Expt. 1. Diurnal variation of (*a*) sucrase (*EC* 3.2.1.48), (*b*) maltase (*EC* 3.2.1.20), (*c*) lactase (*EC* 3.2.1.23), and (*d*) trehalase (*EC* 3.2.1.28) activities in the proximal jejunum of rats on *ad lib.* low-starch (○) or high-starch (●) diets for 14 d. For details of diets, see Table 1. For details of procedures, see pp. 268–269.

diet S for 6 h led to a slight increase in trehalase activity in the morning and afternoon (Table 3). However, there was no significant interaction between diet effect and time effect.

Giving diet S for 12 h to rats initially fed on diet Lst resulted in a significant increase in sucrase, maltase and lactase activities (Table 4) at 10.00 hours and 22.00 hours; trehalase activity was not measured. As observed in the 6 h feeding experiment, the absolute difference in sucrase, maltase, and lactase activities between the two dietary groups as a result of giving diet S for 12 h was identical for each disaccharidase regardless of the time of day when diet S was given (Table 4). Again there was no significant interaction between diet effect and time effect.

Response of sucrase and lactase activities along the villus–crypt columns to dietary carbohydrate

In the subsequent studies, we explored whether the distribution along the villus–crypt columns of enterocytes that responded to diet S was influenced by a diurnal rhythm (Figs 3 and 4). When the rats were force-fed for 6 h and killed at 10.00 hours, sucrase activity in rats fed on diet S was significantly higher in the lower part of the villus (e.g. $P < 0.05$ at 55

Table 2. *Expt. 2*. Sucrase* (EC *3.2.1.48), maltase* (EC *3.2.1.20), lactase* (*EC 3.2.1.23*) *and trehalase* (*EC 3.2.1.28*) *activities*† *in the jejunal mucosa of rats fed on a low-starch or high-starch diet* ad lib. *for 7 d*

(Mean values for four rats per group)

	Sucrase	Maltase	Lactase	Trehalase
Low-starch diet‡				
10.00 hours§	0·87	6·97	0·259	3·86
22.00 hours	1·63	12·24	0·415	3·85
High-starch diet‡				
10.00 hours	3·51	14·48	0·619	3·42
22.00 hours	5·46	29·16	1·105	6·03
SED (12 df)	0·24	0·98	0·055	0·29
Statistical significance of comparisons: P				
Low-starch v. high-starch	< 0·001	< 0·001	< 0·001	< 0·025
10.00 hours v. 22.00 hours	< 0·001	< 0·001	< 0·001	< 0·001
Interaction	< 0·05	< 0·001	< 0·025	< 0·001

SED, standard error of difference.
* For details of procedures, see pp. 268–269.
† Disaccharidase activities are expressed as μmol/mg protein per h.
‡ For details of diets, see Table 1.
§ Time of killing.

Table 3. *Expt. 3*. Sucrase* (EC *3.2.1.48), maltase* (EC *3.2.1.20), lactase* (*EC 3.2.1.23*) *and trehalase* (EC *3.2.1.28) activities*† *in the jejunal mucosa of rats fed on a low-starch diet for 7 d and then force-fed a low-starch diet or a sucrose diet during the last 6 h*

(Mean values for four rats per group)

	Sucrase	Maltase	Lactase	Trehalase
Low-starch diet‡				
10.00 hours§	0·59	4·60	0·344	2·00
22.00 hours	1·60	10·10	0·487	2·43
Sucrose diet‡				
10.00 hours	1·01	6·49	0·491	2·52
22.00 hours	2·05	12·36	0·625	3·00
SED (12 df)	0·12	0·61	0·059	0·22
Statistical significance of comparisons: P				
Low-starch v. sucrose	< 0·05	< 0·01	< 0·05	< 0·05
10.00 hours v. 22.00 hours	< 0·001	< 0·001	< 0·05	< 0·05
Interaction	NS	NS	NS	NS

SED, standard error of difference.
* For details of procedures, see pp. 268–269.
† Disaccharidase activities are expressed as μmol/mg protein per h.
‡ For details of diets, see Table 1.
§ Time of killing.

and 45% of villus–crypt columns) as compared with that of rats on diet Lst (Fig. 3(*a*)). However, lactase activity was elevated following diet S at the upper part of the villus, e.g. $P < 0.01$ at 85, 75 and 65% of villus–crypt columns (Fig. 4(*a*)). In the rats killed at 22.00 hours, the locus of the enterocytes along the villus–crypt columns that responded with an
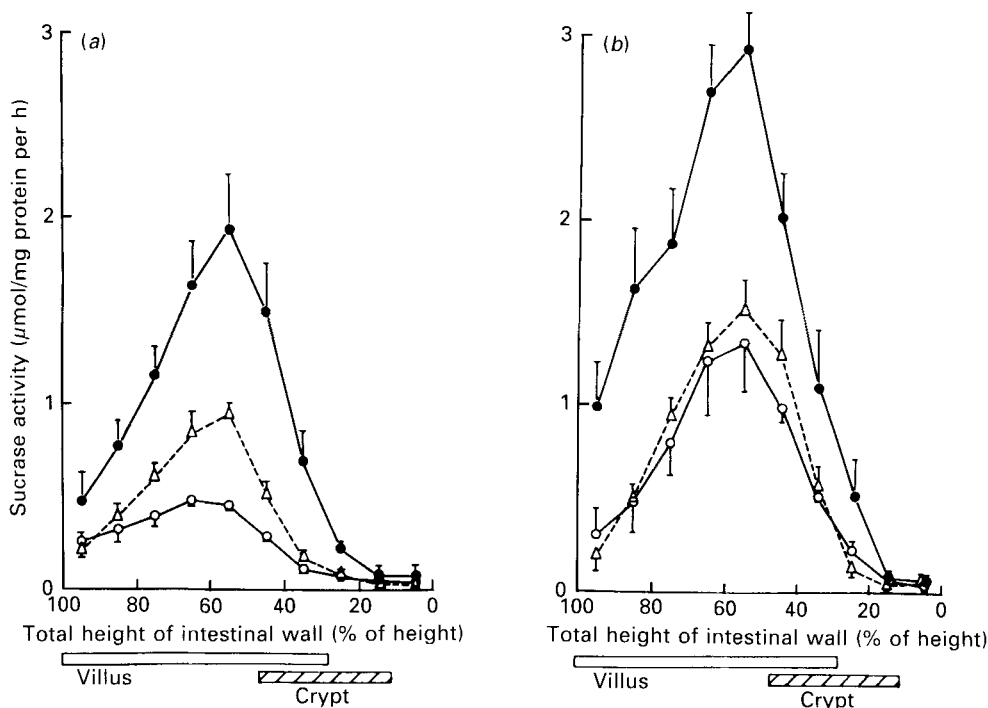
Fig. 3. Expt. 3. Sucrase (*EC* 3.2.1.48) activity along the villus–crypt columns 6 h after the initiation of force-feeding of the sucrose diet. The rats were killed at (*a*) 10.00 hours or (*b*) 22.00 hours. The abscissa shows the total height of the intestinal wall, with 100% representing the apical part of the villus and 0% representing the bottom of the serosal side. (□), Villus region; (▨), crypt region; the overlapping area is the crypt–villus transition (mix) zone. Points are means with their standard errors represented by vertical bars for four rats. (○), Low-starch diet (control); (△), sucrose diet for 6 h; (●), sucrose diet for the entire experimental period.

Table 4. *Expt. 4*. Sucrase (EC 3.2.1.48), maltase (EC 3.2.1.20) lactase (EC 3.2.1.23) activities† in the jejunal mucosa of rats fed on a low-starch diet for 7 d and then force-fed a low-starch diet or a sucrose diet during the last 12 h*

(Mean values for four rats per group)

	Sucrase	Maltase	Lactase
Low-starch diet‡			
10.00 hours§	0·92	8·10	0·573
22.00 hours	1·24	12·71	0·556
Sucrose diet‡			
10.00 hours	2·02	16·01	1·007
20.00 hours	2·31	19·45	0·972
SED (12 df)	0·09	0·84	0·057
Statistical significance of comparisons: *P*			
Low-starch *v.* sucrose	< 0·001	< 0·001	< 0·001
10.00 hours *v.* 22.00 hours	NS	< 0·005	NS
Interaction	NS	NS	NS

SED, standard error of difference.
* For details of procedures, see pp. 268–269.
† Disaccharidase activities are expressed as μmol/mg protein per h.
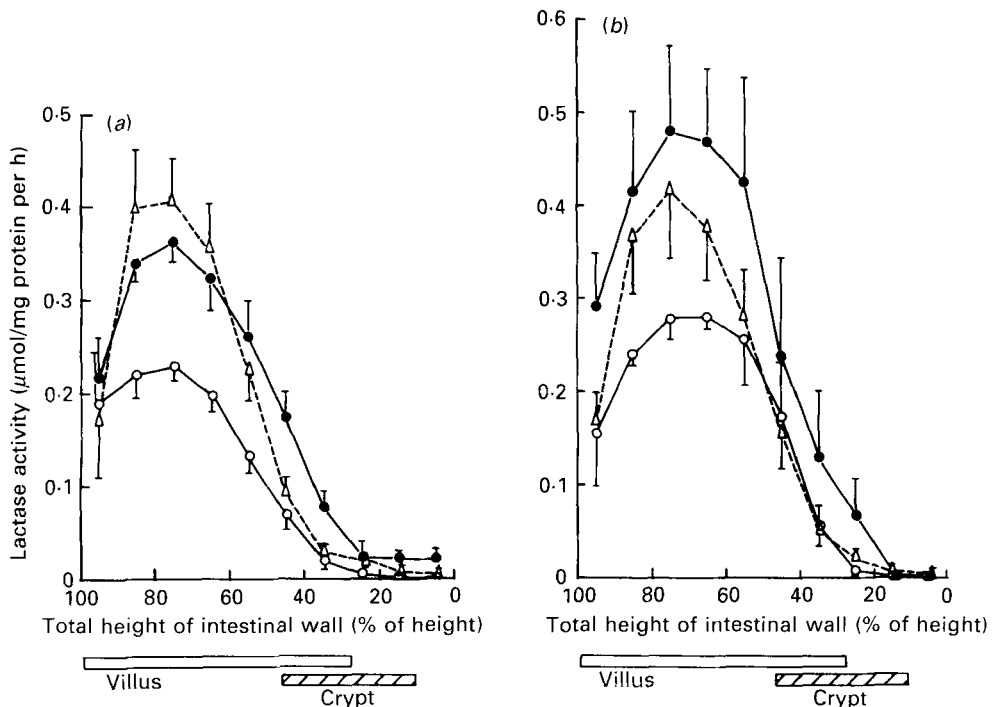‡ For details of diets, see Table 1.
§ Time of killing.

Fig. 4. Expt. 3. Lactase (*EC* 3.2.1.23) activity along the villus–crypt columns 6 h after the initiation of force feeding of the sucrose diet. The rats were killed at (*a*) 10.00 hours or (*b*) 22.00 hours. The abscissa depicts the total height of the intestinal wall, with 100 % representing the apical part of the villus and 0 % representing the bottom of the serosal side. (□), Villus region; (▨) crypt region; the overlapping area is the crypt–villus transition (mix) zone. Points are means with their standard errors represented by vertical bars for four rats. (○), Low-starch diet (control); (△), sucrose diet for 6 h; (●), sucrose diet for the entire experimental period.

increase of sucrase and lactase activity was similar to that seen in the rats killed at 10.00 hours (Figs 3(*b*) and 4(*b*)). Sucrase activity of rats fed on diet S *ad lib*. for 7 d was markedly higher than both groups of rats fed on diet Lst (and force-fed with either diet Lst or diet S for 6 h) along the entire height of the villus regardless of the time of killing (Fig. 3). Similarly, lactase activity of rats fed on diet S *ad lib*. for 7 d was higher than that of rats fed on diet Lst (and then force-fed for 6 h) along the entire height of the villus at both 10.00 and 22.00 hours. However, within 6 h after the initial force-feeding with diet S, lactase activity reached the level seen in the rats fed on diet S for 7 d at the upper part of the villus, but not in the lower part of the villus (Fig. 4).

DISCUSSION

The present study had confirmed and expanded previous studies in this laboratory (Yamada *et al.* 1981*a*; Goda *et al.* 1984, 1985) and others (Cézard *et al.* 1983) which demonstrate that sucrase and lactase activities increase in the rat jejunum within a short time after the introduction of a high-carbohydrate diet. Although sucrase activity reaches a maximal level 24–48 h after the introduction of a sucrose-containing diet (Goda *et al.* 1985), feeding with diet S led to a marked increase in sucrase and lactase activities within 12 h; this increase was detectable as early as 6 h following the change to a high-carbohydrate diet.

This dietary effect was observed not only when disaccharidase activities were decreasing to minimum levels (i.e. between 22.00 and 10.00 hours), but also when they were increasing

to peak levels (i.e. between 10.00 and 22.00 hours). The absolute increases in sucrase, maltase and lactase activities induced by feeding with diet S before 10.00 hours were identical to those induced by giving the same amount of diet S before 22.00 hours. A two-way analysis of variance revealed that there was no significant interaction between the diet effect and the time effect, which suggested a constant (time-independent) effect of diet S. These results indicate that small intestinal epithelial cells retained the capability to respond to a dietary modification of carbohydrate at any time of the day, and that there was no diurnal variation of the 'adaptational' capability.

The lack of a diurnal variation of 'adaptational' capability of the enterocytes was further supported by analysis of the activities of lactase and sucrase along the height of the villus–crypt columns. The present findings showed that the distribution of cell cohorts that responded to the dietary manipulation with an increase in lactase and sucrase activities to the dietary manipulation was not affected by the time of introduction of diet S. Furthermore, the present results are in agreement with a previous report (Goda *et al.* 1985), which revealed that the lower part of the villus is the major reactive site of sucrase activity, whereas the upper villus is the major reactive site of lactase activity.

The present study also revealed that a typical diurnal variation of small intestinal sucrase, maltase, and lactase activities (Saito, 1972; Stevenson *et al.* 1975) was seen in rats fed on diet Lst as well as in rats fed on diet Hst. It is interesting to note that the increase in disaccharidase activity between 10.00 and 22.00 hours is much greater on the high-starch diet than the low-starch diet. It is very likely that the extent of the diurnal variation of disaccharidase activities is affected by the amount of carbohydrate consumed. However, the pattern of diurnal variation (e.g. percentage changes) of disaccharidase activities was similar for the two dietary groups (Table 2). This result suggests that the pattern of diurnal variation of intestinal disaccharidase activities persists regardless of the amount of carbohydrate consumed. This is supported by the report of Saito *et al.* (1978) who demonstrated that diurnal variation of jejunal sucrase and maltase activities was seen even in rats fed on a carbohydrate-free diet. Thus, either the protein intake (Furuya *et al.* 1979) or the energy intake, rather than the carbohydrate intake, may be a major cue in maintaining a basal diurnal variation of disaccharidase activities.

Since our objective was to analyse quantitatively the capability of intestinal epithelial cells to respond to dietary carbohydrate at different times of the day, it was necessary to control food intake strictly. This was done by a force-feeding procedure for which we have previously established that lactase or sucrase activities, villus height and cell migration rates are not modified (Goda *et al.* 1984, 1985). However, since it is known that meal-feeding restricted to a short period of time (3–6 h/d) can cause a shift of peak activity of intestinal disaccharidases to the time of feeding (Saito *et al.* 1975, 1976), it was necessary to select the shortest time-period (6 h) of dietary manipulation that led to a profound effect on disaccharidase activities (Goda *et al.* 1985). In this regard, it is interesting to note that 6 h after the initial force-feeding of diet Lst, a clear diurnal variation of sucrase and maltase activities was still observed, whereas the diurnal variation of lactase activity was obscured (see Table 3). At 12 h, the diurnal variation of sucrase and maltase activities remained, though it was less apparent, and the diurnal variation of lactase activity was completely abolished (see Table 4). These results suggest that (*a*) the interruption of a diurnal variation of food intake caused by intermittent force-feeding of a constant amount of diet might lead to the disappearance of the diurnal variation of intestinal disaccharidase activities within a relatively short period of time (i.e. 6–12 h), and (*b*) the diurnal variation of lactase activity might be influenced by dietary manipulation in a shorter time than sucrase and maltase activities.

It should be noted that not only the activities of sucrase and maltase (α-glucosidases), but

also the activity of lactase (a β-galactosidase), was affected by feeding with diet S, and that lactase activity was modified according to diurnal variation. In contrast, the activity of another α-glucosidase, trehalase, appeared to be regulated in a manner, distinct from the other disaccharidases. Trehalase activity exhibited a diurnal variation only when rats were fed on diet Hst; no diurnal variation was detected in rats fed on diet Lst. Furthermore, trehalase activity is dependent on carbohydrate intake only when it is measured at a time when diet consumption is high. This observation supports the results of Shinohara et al. (1986), who showed no effect of carbohydrate intake on trehalase activity in rats killed in the morning.

The mechanism by which the diurnal variation of small intestinal disaccharidase activities is expressed is not clear. Kaufman et al. (1980) have proposed that the diurnal variation of sucrase activity is based on a variation in the rate of degradation, as opposed to the rate of synthesis, of sucrase–isomaltase. In their report Kaufman et al. (1980) showed that the rate of degradation of sucrase was much slower when a carbohydrate-containing diet was provided than when the diet was removed. Since many investigators have demonstrated that pancreatic secretions play a role in the degradation of sucrase–isomaltase and lactase (Alpers & Tedesco, 1975; Goda & Koldovský, 1985; Riby & Kretchmer, 1985; Goda et al. 1988), it is conceivable that a cyclic pattern of pancreatic protease(s) could be responsible for diurnal variation of disaccharidase activities. However, no diurnal variation of lumen trypsin activity was observed in rats fed on a standard laboratory diet (Goda & Koldovský, 1985) or in those fed on diets Lst or Hst (present report). This suggests that the secretion or activation of trypsinogen, or both, is not involved in the expression of a diurnal variation of disaccharidase activities. This agrees with the lack of cyclic change of pancreatic content of trypsinogen (George et al. 1985) and absence of cyclic change of rate of synthesis of trypsinogen in the pancreas (Girard-Globa et al. 1980). The involvement of other proteases (e.g. chymotrypsin (EC 3.4.21.1), elastase (EC 3.4.21.36), however, cannot be excluded at present.

In contrast to the diurnal variation of sucrase activity, the dietary-induced increase in sucrase activity has been shown to occur with a concomitant increase in the rate of synthesis of sucrase–isomaltase in rats previously fed on a carbohydrate-free diet (Cézard et al. 1983). It has also been shown that in rats fed on a high-carbohydrate diet for a few days, the rate of incorporation of a precursor amino acid into sucrase–isomaltase (Riby & Kretchmer, 1984; Tsuboi et al. 1985), lactase (Tsuboi et al. 1985) and glucoamylase (EC 3.2.1.3) (Tsuboi et al. 1985) is increased, compared with rats fed on a low-starch diet. Further studies are needed to explore the molecular mechanisms involved in these two apparently independent variations in microvillar disaccharidase activities in the small intestine.

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