Effects of Diets on Hydrolase Activities Localized in the Brush Border Membrane of the Small Intestine in Rats

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This study was undertaken to investigate the response of sucrase (SA), alkaline phosphatase (ALP), and leucine aminopeptidase (LAP) activities localized in the brush border membrane of the small intestine to ingested nutrients. In rats previously meal-fed on carbohydrate-free diets, the maximal increase in SA activity after the administration of a 18% casein and high sucrose diet (HSD) occurred 24 hr after the beginning of the 12-hr period of HSD-feeding, but ALP and LAP activities reached the maximal levels 12 hr after the HSD administration and then rapidly declined. These changes in three enzyme activities were similar to those after HSD-feeding following a 2-day fast, but those were not observed after giving a carbohydrate-free and high fat diet. The increases in these enzyme activities after giving HSD were not observed entirely after giving HSD containing 0.5% concanavalin A, which was undigestible and preferentially bound mitotically active cells of the small intestine. From these findings, it can be suggested that the adaptive response of these enzyme activities to ingested nutrients, as sucrose and casein, was produced on the luminal surface of the small intestine, especially on the immature cells.

Many investigators1 ~ 3) have reported that rat intestinal sucrase, alkaline phosphatase, and leucine aminopeptidase activities are localized in the brush border membrane of the small intestine. In rats given a high sucrose diet following a 2- or 3-day fast, jejunal alkaline phosphatase and leucine aminopeptidase activities rapidly increased just after refeeding, and reached the maximal levels 12 hr after giving the diet.4 5) On the other hand, sucrose-mediated changes in jejunal sucrase activity in these rats were initiated at the level of the crypt cell and were expressed after a latent period of 18 to 20 hr, during which these cells matured and migrated toward the villus tip.4 6 ~ 8) Cezard et al.,9 10) thereafter, reported that the maximal increase in sucrase activity caused by sucrose feeding in rats given a carbohydrate-free diet occurred by 12 hr after giving the sucrose diet, and separation of villus and crypt cells 12 hr after giving the diet revealed a parallel increase of sucrase activity along the villus-crypt axis. They proposed that the sucrose-mediated increase in sucrase activity, rather than acting preferentially on the crypt, occurred over the entire villus-crypt unit. This study was undertaken to observe the course of changes in jejunal sucrase, alkaline phosphatase, and leucine aminopeptidase activities after giving a high sucrose diet or a high fat diet in rats previously fed on a carbohydrate-free diet, and to investigate the response of these enzyme activities to ingested nutrients.

Materials and Methods

Animals. Male rats of the Wistar strain weighing approximately 100 g were obtained from JAPAN SLC Inc., Shizuoka, Japan. They were individually housed in suspended wire-mesh cages in a room kept at 21 to 23°C with lighting regulated to provide automatically a 12-hr light period (0800 ~ 2000 hr), and a 12-hr dark period (2000 ~ 0800 hr). Rats were fed on a cereal based stock diet (F-2, Funabashi Farm, Chiba, Japan) in a 12-hr feeding period (1900 ~ 0700 hr) for 7 days before the start of the experiments except for experiments 2 and 5.

Diets. Two carbohydrate-free diets and a high sucrose
diet were prepared. One of the carbohydrate-free diets was the high fat diet (HFD), the composition of which was similar to that adopted by Cezard et al.9); 18% casein, 34% vegetable oil, 43% a-cellulose (Sigma Chemical Co., St. Louis, U.S.A.), 4% mineral mixture,11} and 1% vitamin mixture. The other was the high casein diet (HCD); 84% casein, 10% vegetable oil, 4% mineral mixture, and 2% vitamin mixture. The composition of the high sucrose diet (HSD) was similar to that of the diet used by Cezard et al.9); 18% casein, 67% sucrose, 10% vegetable oil, 4% mineral mixture, and 1% vitamin mixture.

Experiment 1. Rats were given HFD from 1900 hr to 0700 hr for 3 days. On the following day, they were placed four each into four groups. Rats in group 1 were killed at 1900 hr, and the others were given HSD from 1900 hr to 0700 hr. Rats in groups 2 to 4 were killed 6, 12, or 24 hr, respectively, after being given HSD. Immediately after killing, a segment of jejunum, representing the second 15-cm segment distal from the pylorus, was excised. This segment was slit longitudinally after being rinsed with cold saline and homogenized in distilled water using an Ultra Turrax homogenizer (Ika Werk, Staufen, West Germany). The resulting homogenate was used for measurement of enzyme activities and protein.

Experiment 2. Rats were given HFD from 1900 hr to 0700 hr for 3 days. On the following day, they were divided four each into 10 groups. Rats in group 1 were killed at 1900 hr, and those in groups 2 to 7 were given HSD or HFD from 1900 hr to 0800 hr and killed 6, 12, or 24 hr, respectively, after being given HSD or HFD. Rats in groups 8 to 10 were not given any diet, and were killed when those in group 2 to 7 were killed. The small intestine was treated by a procedure similar to that in the previous experiment.

Experiments 3 and 4. Rats were given HFD in experiment 3 or HCD in experiment 4 from 1900 hr to 0700 hr for 3 days. On the following day, they were given HSD or HSD containing 0.5% concanavalin A (Con A, type IV, Sigma Chemical Co., St. Louis, U.S.A.) respectively, at 1900 hr, and they were killed just before and 12 or 24 hr after being given the diet. The small intestine was treated as described in experiment 1 for measurement of enzyme activities and protein.

Experiment 5. Rats were fed HSD ad libitum for 3 days. Thereafter, they were fed HSD ad libitum, HSD containing 0.5% Con A, HFD, or HFD containing 0.5% Con A, and they were killed at 1000 hr on the 4th day of these feedings. The small intestine was treated as mentioned in the previous experiments.

Analytical procedure. Sucrase (SA) activity was measured by the method of Dahlqvist.13) Alkaline phosphatase (ALP) activity was measured by the method of King and Kind.14) Leucine aminopeptidase (LAP) activity was measured by the method of Goldberg et al.15) These enzyme activities were expressed in terms of micromoles of substrate hydrolyzed per hr unit length of the small intestine (designated as the segmental activity) as described in our previous studies.4'5} Protein was measured by the method of Lowry et al.16} using bovine serum albumin as a standard.

Statistical analysis. Statistically significant differences among treatment means were identified by the least significant difference calculated by the method of Snedecor and Cochran.17)

Results

As shown in Table I, in experiment 1, there was no difference between the amounts of consumed food in rats killed 12 hr after being given HSD and those in rats killed 24 hr after being given the diet, but those in these rats were significantly higher than those in rats killed 6 hr after giving HSD. The segmental weight and protein content of the small in-

Table I. Effects of a High Sucrose Diet (HSD)-Feeding on Small Intestine in Rats Previously Meal-Fed a High Fat Diet (Experiment 1)

<table>
<thead>
<tr>
<th>Hours of after beginning of HSD-feeding</th>
<th>0</th>
<th>6</th>
<th>12</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight1 g</td>
<td>119 ± 2.02a</td>
<td>119 ± 2.3a</td>
<td>118 ± 4.3a</td>
<td>115 ± 1.6a</td>
</tr>
<tr>
<td>Food consumed g</td>
<td>0</td>
<td>7.0 ± 0.5a</td>
<td>10.1 ± 0.4b</td>
<td>10.7 ± 0.3b</td>
</tr>
<tr>
<td>Small intestine (SI) mg/segment³</td>
<td>606 ± 3.8a</td>
<td>627 ± 19.1a</td>
<td>653 ± 27.7a</td>
<td>632 ± 24.2a</td>
</tr>
<tr>
<td>SI protein mg/g SI</td>
<td>132 ± 2.2a</td>
<td>130 ± 3.0a</td>
<td>131 ± 1.5a</td>
<td>145 ± 6.5a</td>
</tr>
<tr>
<td>SI protein mg/segment</td>
<td>80 ± 1.2a</td>
<td>81 ± 2.8a</td>
<td>85 ± 3.9a</td>
<td>84 ± 6.6a</td>
</tr>
</tbody>
</table>

1 Before HSD-feeding.
2 Mean ± SE (N = 4): means in the same row with unlike superscript letters are significantly different (p < 0.05).
3 The second 15-cm segment from the pylorus.
Diets and Intestinal Hydrolase Activities of Rats

Fig. 1. Effects of a High Sucrose Diet (HSD)-feeding on Intestinal Sucrase (SA), Alkaline Phosphatase (ALP), and Leucine Aminopeptidase Activities in Rats Previously Meal-fed a High Fat Diet (Experiment 1). These enzyme activities are expressed as a percentage of the activities in rats just before HSD-feeding. In rats just before HSD-feeding, these activities (micromoles of substrate hydrolyzed per hr in the second 15-cm segment from the pylorus) were as follows: SA, 167 ± 6; ALP, 4220 ± 410; LAP, 93 ± 3. Vertical bars represent the SE of the mean for four rats.

Fig. 2. Effects of Starvation or Diet-feeding on Intestinal Sucrase (SA), Alkaline Phosphatase (ALP), and Leucine Aminopeptidase Activities in Rats Previously Meal-fed a High Fat Diet (Experiment 2). These enzyme activities are expressed as a percentage of the activities in rats just before starvation or a high sucrose diet (HSD)- and a high fat diet (HFD)-feeding. In rats just before starvation or HSD- and HFD-feeding, these activities (micromoles of substrate hydrolyzed per hr in the second 15-cm segment from the pylorus) were as follows: SA, 359 ± 15; ALP, 5170 ± 610; LAP, 389 ± 12. X-X, starved; O-O, HSD; ●-●, HFD. Vertical bars represent the SE of the mean for four rats.

testine were statistically no difference among the four groups. The segmental SA activity increased gradually for 24 hr after giving HSD, but ALP and LAP activities reached the maximal levels 12 hr after giving HSD, and then rapidly declined (Fig. 1). The results of experiment 2 are shown in Fig. 2. The course of changes in these three enzyme activities after giving HSD were similar to those observed in experiment 1, but LAP and ALP activities reached the maximal levels 6 hr after giving HSD. On the other hand, those in these three enzyme activities in rats given HFD or not given any diet during the feeding period were not definitely observed for 24 hr after the beginning of the feeding period. The results of experiment 3 are shown in Table II and Fig. 3. The amounts of food consumed in rats given HSD containing 0.5% Con A were about 80% of those in rats given HSD. The segmental
Table II. Effects of Feeding a High Sucrose Diet (HSD) Containing Concanavalin A (Con A) on Small Intestine in Rats Previously Meal-Fed a High Fat Diet (Experiment 3)

<table>
<thead>
<tr>
<th>Experimental diet</th>
<th>Hours after beginning of diet-feeding</th>
<th>HSD</th>
<th>HSD + 0.5% Con A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Body weight g</td>
<td></td>
<td>107 ± 2.0 a</td>
<td>104 ± 1.4 a</td>
</tr>
<tr>
<td>Food consumed g</td>
<td></td>
<td>0</td>
<td>10.3 ± 0.4 a</td>
</tr>
<tr>
<td>SI mg/segment</td>
<td></td>
<td>677 ± 26 a</td>
<td>756 ± 22 b</td>
</tr>
<tr>
<td>SI protein mg/g SI</td>
<td></td>
<td>141 ± 2.5 ab</td>
<td>141 ± 5.2 ab</td>
</tr>
<tr>
<td>SI protein mg/segment</td>
<td></td>
<td>96 ± 2.3 ab</td>
<td>107 ± 5.1 a</td>
</tr>
</tbody>
</table>

1 Before experimental diet-feeding.
2 Mean ± SE (N = 4); means in the same row with unlike superscript letters are significantly different (p < 0.05).
3 The second 15 cm segment from the pylorus.

Fig. 3. Effects of Feeding a High Sucrose Diet (HSD) Containing Concanavalin A (CA) on Intestinal Sucrase (SA), Alkaline Phosphatase (ALP), and Leucine Aminopeptidase (LAP) Activities in Rats Previously Meal-Fed a High Fat Diet (Experiment 3).

These enzyme activities are expressed as a percentage of the activities in rats just before HSD-feeding. In rats just before HSD-feeding, these activities (micromoles of substrate hydrolyzed per hr in the second 15 cm segment from the pylorus) were as follows: SA, 224 ± 15; ALP, 6900 ± 1350; LAP, 268 ± 16. Vertical bars represent the SE of the mean for four rats.

weight and protein content of the small intestine were slightly greater in rats given HSD than in rats given HSD containing 0.5% Con A. Changes in SA, ALP, and LAP activities after giving HSD in this experiment were observed to be similar to those in the previous experiments, but the marked increases in these enzyme activities after giving HSD were completely prevented by the addition of 0.5% Con A to HSD. The results of experiment 4 are shown in Table III and Fig. 4. Effects of HSD-feeding on the amounts of food consumed, the segmental weight and protein content, and SA, ALP, and LAP activities of the small intestine in rats previously fed on HCD were the same as those in rats previously fed HFD in experiment 3. And, as observed in the previous experiment, the addition of 0.5% Con A to HSD absolutely prevented the significant increases in these three enzyme activities after HSD-feeding. Table IV and Fig. 5 show the results of experiment 5. There were statistically no differences in the amounts of food consumed and body weight gain between rats given HSD and rats given HFD, but the addition of 0.5% Con A to these diets caused a marked reduction in the amounts of food consumed and body weight gain. Liver weights of rats given HSD were appreciably higher than those of rats given HFD, and the addition of 0.5% Con A to these diets markedly reduced the liver weight. The segmental weight and
Table III. Effects of Feeding a High Sucrose Diet (HSD) Containing Concanavalin A (Con A) on Small Intestine in Rats Previously Meal-Fed a High Casein Diet (Experiment 3)

<table>
<thead>
<tr>
<th>Experimental diet</th>
<th>HSD</th>
<th>HSD + 0.5% Con A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hours after beginning of diet-feeding</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td><strong>Body weight</strong></td>
<td>106 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>110 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Food consumed</strong></td>
<td>0</td>
<td>12.1 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Small intestine (SI)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg/segment</td>
<td>642 ± 18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>773 ± 12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SI protein mg/g SI</td>
<td>135 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>134 ± 5.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>mg/segment</td>
<td>87 ± 2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>105 ± 3.6&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1 Before experimental diet-feeding.
2 Mean ± SE (N=4); means in the same row with unlike superscript letters are significantly different (p < 0.05).
3 The second 15cm segment from the pylorus.

Fig. 4. Effects of Feeding a High Sucrose Diet (HSD) Containing Concanavalin A (CA) on Intestinal Sucrase (SA), Alkaline Phosphatase (ALP), and Leucine Aminopeptidase (LAP) Activities in Rats Previously Meal-Fed a High Casein Diet (Experiment 4).

These enzyme activities are expressed as a percentage of the activities in rats just before HSD-feeding. In rats just before HSD-feeding, these enzyme activities (micromoles of hydrolyzed per hr in the second 15-cm segment from the pylorus) were as follows: SA, 267 ± 9; ALP, 4010 ± 650; LAP, 289 ± 12. Vertical bars represent the SE of the mean for four rats.

The protein content of the small intestine were not significantly affected by the addition of 0.5% Con A to these diets. SA, ALP, and LAP activities in rats given HSD were significantly higher than those in rats given HFD, and the addition of 0.5% Con A to these diets reduced these enzyme activities. These effects of diets and Con A to the diets, however, on SA activity were more definitely observed than those on ALP and LAP activities.

Discussion

Cezard et al.<sup>9,10</sup> demonstrated that the maximal increase in jejunal SA activity caused by a high sucrose diet in rats fed on a carbohydrate-free and high fat diet occurred within 12 hr after giving the high sucrose diet. In these studies, however, the description of the experimental procedure for changes in SA activity was as follows. “Rats were fed from 7:00 PM to 7:00 AM on the diet… Animals were then killed at varying time periods from 12 to 60 hr after the beginning of the diet, but always between 7:00 AM and 8:00 AM, in order to standardize for the known circadian rhythm of sucrase.” In this experimental procedure, the varying time periods are limited to only 12, 36, and 60 hr after the beginning of diet-feeding, and accordingly the data obtained at the time periods except the above-mentioned time periods, especially the 24 hr period, throw doubt
Table IV. Effects of Feeding a High Sucrose Diet (HSD) or a High Fat Diet (HFD) Containing Concanavalin A (Con A) on Small Intestine and Liver Weight in Rats Previously Fed on HFD (Experiment 5)

<table>
<thead>
<tr>
<th>Experimental diet</th>
<th>HSD</th>
<th>HSD+0.5%Con A</th>
<th>HFD</th>
<th>HFD+0.5%Con A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight g</td>
<td>102 ± 3.4a</td>
<td>99 ± 5.3a</td>
<td>101 ± 5.4a</td>
<td>99 ± 7.0a</td>
</tr>
<tr>
<td>Body weight gain g/3 days</td>
<td>12.8 ± 2.3a</td>
<td>5.5 ± 1.7b</td>
<td>14.2 ± 1.0a</td>
<td>3.8 ± 0.6b</td>
</tr>
<tr>
<td>Food consumed g</td>
<td>11.9 ± 0.7a</td>
<td>8.9 ± 0.8a</td>
<td>12.0 ± 0.2a</td>
<td>8.4 ± 0.2b</td>
</tr>
<tr>
<td>Liver weight g</td>
<td>6.27 ± 0.16a</td>
<td>4.96 ± 0.33b</td>
<td>4.54 ± 0.16b</td>
<td>3.79 ± 0.26a</td>
</tr>
<tr>
<td>Small intestine (SI) mg/segment²</td>
<td>779 ± 22a</td>
<td>750 ± 24a</td>
<td>817 ± 20a</td>
<td>778 ± 30a</td>
</tr>
<tr>
<td>Si protein mg/g SI</td>
<td>142 ± 3.8a</td>
<td>132 ± 1.8b</td>
<td>140 ± 2.4ab</td>
<td>138 ± 2.3b</td>
</tr>
<tr>
<td>mg/segment</td>
<td>111 ± 2.1ab</td>
<td>99 ± 4.4a</td>
<td>115 ± 3.8b</td>
<td>107 ± 3.4ab</td>
</tr>
</tbody>
</table>

1 Mean ± SE (N = 5): means in the same row with unlike superscript letters are significantly different (p < 0.05).
2 The second 15-cm segment from the pylorus.

Fig. 5. Effects of Feeding a High Sucrose Diet (HSD) or a High Fat Diet (HFD) Containing Concanavalin A (CA) on Intestinal Sucrase (SA), Alkaline Phosphatase (ALP), and Leucine Aminopeptidase (LAP) Activities in Rats Fed on HFD (Experiment 5).

Vertical bars represent the SE of the mean for four rats.

sucrose. In rats given HSD following a 2- or 3-day fast, significant increases in SA activity after giving HSD occurred after a latent period of 16 to 20 hr. Cezard et al. suggested that the delayed increase in SA activity after giving HSD following a 2- or 3-day fast was produced by a lack of response of upper villus cells to substrate stimulation after a prolonged fast. As shown in Figs. 1 and 2, SA activity in rats previously fed on the carbohydrate-free diets significantly increased in a relatively short period after giving HSD. These findings were compatible with those reported by Cezard et al. They demonstrated that there was no difference in SA activity between 12 hr and 36 hr after giving HSD, and that the maximal increase in SA activity occurred by 12 hr after giving HSD. In this study, the maximal increase in SA activity by HSD-feeding was expressed 24 hr after giving the diet (Figs. 1 to 4). As shown in Fig. 2, SA activities after giving HFD or by starvation maintained the constant levels. These findings demonstrated that the change in SA activity was not affected by the circadian rhythm, the maximal increase in SA activity was produced every 24 hr after giving HSD, and significant increases in SA activity after giving HSD were mediated by ingested sucrose.
creased just after giving HSD, and reached the highest levels at the final stage of the feeding period (Figs. 1 to 4). The maximal levels of these enzyme activities were observed 12 hr in experiment 1 and 6 hr in experiment 2, after giving HSD. These differences may be caused by the low amounts of food consumed in experiment 2, which was initiated without a daily 12-hr feeding period giving the stock diet for 7 days, compared with those in experiment 1. The changes in ALP and LAP activities after giving HSD in rats previously fed on the carbohydrate-free diets showed close agreement with those after giving HSD to starved rats.4,5 Changes in ALP and LAP activities for 24 hr after giving HFD or starvation were insignificant compared with those after giving HSD (Fig. 2). Cassidy et al.19) reported that the mucosa of the cellulose-feeding rats did not morphologically differ from the control rats. In our study, HFD administration during the previous feeding period caused no adverse effect on food consumed or body weight gain. Therefore, the significant increases in ALP and LAP activities, as well as SA activity, after giving HSD were demonstrated to be mediated by ingested HSD. In experiments 1 to 4, effects of diets in meal-feeding on SA, ALP, and LAP activities were observed, and also, in experiment 5, those with ad libitum feeding were observed. The results of experiment 5 (Fig. 5) indicated that there was no substantial difference in effects of diets on these enzyme activities between the meal feeding and the ad libitum feeding.

As shown in Table IV, liver weights in rats given HSD were significantly higher than those in rats given HFD, and the addition of the small amount of Con A to HSD or HFD significantly reduced those in rats administered these diets, respectively. These observations indicated that liver weights were affected by only the amount of food consumed, but also the composition of diets. The effects of diet-composition on liver weights were in common with those on SA, ALP, and LAP activities (Fig. 5). In our previous study,5) LAP activity in rats given a 89% casein diet following a 2-day fast was observed to reach a significantly high level 12 hr after giving the diet. From these findings, the adaptive increases in these SA, ALP, and LAP activities were suggested to be induced by the dietary components to be digested and absorbed in the small intestine, and transported to the liver via the portal vein; as sucrose and casein.

In our previous study,5) we found that the adaptive increases in SA, ALP, and LAP activities in rats after giving HSD following a 2-day fast were completely restricted with an addition of 0.5% Con A to HSD. The adverse effects of Con A added to HSD on the adaptive changes in these enzyme activities were similarly observed in rats giving HSD after a carbohydrate-free diet (Figs. 3 to 5). A number of toxic bean lectins have been recognized to bind to epithelial cells of different regions of the small intestine in vitro or in vivo, depending on the specificity of the lectins.20~23) In previous studies,5,24) we reported that behavior of ingested Con A in the gastrointestinal tract was as follows. Ingested Con A inertly passed through the lumen of the stomach. The Con A entering the lumen of the small intestine, bound to the luminal surface, and thereafter the bound Con A naturally exfoliated together with mature epithelial cells. Then the Con A was unaltered and excreted in the feces. And the experimental results5) excluded the possibility that the Con A caused the accelerating loss of the brush border membrane. Furthermore, Padolsky and Weiser22) demonstrated that Con A preferentially bound mitotically active cells in the epithelial cells of the small intestine. As mentioned above, the maximal increase in SA activity by sucrose-feeding in rats starved or fed carbohydrate-free diets occurred 24 hr after the beginning of HSD-feeding, during which the crypt cell matured and migrated toward the villus tip.7) SA activities in rats given HSD or HFD containing Con A were reduced to 25% levels of those in rats given HSD or HFD (Fig. 5). Although there were no differences in ALP and LAP activities between rats given HSD containing Con A and rats given HFD containing Con A,
these enzyme activities in rats given these diets were significantly lower than those in rats given the diets without Con A. These enzyme activities are known to be highest in the upper villus zone and almost absent in the crypts.\textsuperscript{25,26} Vasseur \textit{et al.},\textsuperscript{27} however, demonstrated that SA was imbedded superficially in the brush border membrane, and ALP and LAP were buried in the membrane. Accordingly, these enzymes were suggested to be not directly affected by ingested nutrients and Con A.

From these findings and discussion, the following conclusions may be drawn; 1) the primary site for the response of the adaptive changes in these enzyme activities to ingested nutrients was on the luminal surface of the small intestine, especially on the immature cells. 2) The changes in these enzyme activities were produced by those in the respective enzyme formation, which of SA and the others proceeded in the immature cells and in the mature cells, respectively, through the intracellular stimuli from the response.

\textbf{References}