Effect of Feeding Amaranth (Food Red No. 2) on the Jejunal Sucrase and Digestion-Absorption Capacity of the Jejunum in Rats

Hidetoshi TAKEDA* and Shuhachi KIRIYAMA**

Department of Agricultural Chemistry, Faculty of Agriculture, Ehime University, Tarumi 3-5-7, Matsuyama 790, Japan

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Summary To clarify the effect of feeding 5% amaranth (Food Red No. 2, Am) alone or with 5% dietary fiber on jejunal mucosal integrity, change in jejunal sucrase activity before and after the feeding was compared between rats fed and fasted previously. Digestion-absorption capacity of the jejunum was also examined by perfusing 15 mmol/liter sucrose and 30 mmol/liter glycylglycine through the anesthetized rat jejunum after 14 days of feeding Am. Gobo dietary fiber (GDF) was prepared from the roots of edible burdock (Arctium lappa L.). At the end of 3 days' fasting, rats had 20% less body weight, 30% less mucosal protein and 50% less jejunal sucrase activity per unit length than those before fasting. Although rats fed Am showed severe diarrhea and growth retardation as observed in previous reports, initial sucrase level was not changed by feeding Am for 3 days even in the fasted rats. When sucrase activity on day 3 after feeding was compared among inter-groups, however, rats fed Am showed sucrase activity lower than that of rats fed either the basal diet or the basal diet containing Am plus GDF only when they had been fasted previously. After 14 days of feeding, rats fed Am after 3 days' fasting regained sucrase activity up to that of rats fed the basal diet despite the remarkable growth retardation. Jejunal perfusion in situ showed that digestion-absorption capacity for sucrose and glycylglycine in rats fed 5% Am for 14 days was also the same as that in rats fed the basal diet. These results suggest that feeding Am can reduce neither jejunal sucrase nor digestion-absorption capacity of epithelial cells of the jejunum, but retards the regain of the lowered sucrase level at earlier stage of feeding when rats have been fasted before the feeding, and that concurrent feeding of GDF promotes catch-up of the sucrase level.

* Present address: Imabari Meitoku Junior College, 688 Yatako, Imabari 794, Japan.
** Present address: Department of Agricultural Chemistry, Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan.
lowered by the fasting.

**Key Words** dietary fiber, amaranth (Food Red No. 2), sucrate response in fed and fasted rat, jejunal mucosal integrity

Feeding of a purified basal diet containing 5% Am to rats causes severe diarrhea and reductions in food efficiency and growth rate. These adverse effects of Am are completely protected by the concurrent feeding of various dietary fibers at levels of 3 to 10% of diet (1-3). Ershoff (4,5) suggests that the capacity of dietary fiber to bind Am may be a main factor counteracting the Am “toxicity.” He introduced this speculation from an observation that anion-exchange resin such as cholestyramine was protective against Am toxicity in rats (4). However, we found that GDF and its derivatives having anti-Am activity did not adsorb sufficient amount of Am to account for their beneficial effects (6).

On the other hand, Kimura et al. (7) reported that rats fed the basal diet with 5% Am for 4 days after 2 days' fasting had lower levels of jejunal membrane enzymes such as sucrate [EC 3.2.1.26] and alkaline phosphatase [EC 3.1.3.1] than those in rats fed either the basal diet or the basal diet containing 5% Am plus 5% GDF. From these results they suggested that the lowered sucrate activity is attributable to exfoliation of the brush border membrane by Am and GDF exerts its beneficial effect by protecting brush border membrane from the exfoliation.

If Am, as suggested by Kimura et al. (7), exfoliates brush border membranes and thereby sucrate is lost, jejunal sucrate level should continue to decrease as long as the Am feeding is continued. Furthermore, according to “the exfoliation hypothesis” proposed by them, animals fed an Am-containing diet should consistently have the lowered jejunal sucrate level whether or not they had been fasted before the feeding. To confirm these possibilities, we examined in the present study the time-dependent changes in mucosal sucrate activity after rats were fasted for 3 days, and then fed the 3 kinds of diets (the basal, basal plus 5% Am, and basal plus 5% Am plus 5% GDF) over 14 days, and data obtained from these rats were compared with those from rats fed the same diets without fasting before feeding. In addition, we examined digestion-absorption capacity of the jejunum by perfusing jejunal segment in situ with the medium containing sucrose and glycylglycine (hereafter referred to as gly-gly) in rats fed with 5% Am for 14 days.

The results showed that response of sucrate activity when Am was fed to fasted rats was quite different from that when it was fed to non-fasted rats, especially at early stage of feeding. The causes of difference in jejunal sucrate response between the fed and fasted rats are discussed.

**MATERIALS AND METHODS**

**Materials.** We prepared dietary fiber from the roots of edible burdock, which

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is one of the common vegetables available all the year round in Japan and which was reported to contain 3.8% dietary fiber (raw weight basis) (8) as determined by the method of Prosky et al. (9). The procedure of GDF preparation was the same as that described previously (6). Briefly, the edible portion of fresh roots was finely mashed through a disposer (ISE IN-SINK-ERATOR, Model 333SS, Div. Emerson Electric Co., Racine, WI) with running water. After thorough leaching with running water, the residue was boiled in water to remove starches and other water-soluble materials, and extracted and dehydrated by the addition of 99% ethanol and dried in air. The resultant dry residue (yield from fresh roots was about 4%) was powdered in a Wiley mill with a 1 mm diameter pore sieve and used in feeding experiments (referred to as GDF). Chemical composition of GDF is shown in Fig. 1.

Am, trisodium salt of 1-(4-sulfo-1-naphthylazo)-2-naphthol-3,6-disulfonic acid (San-Ei Chemical Industries, Osaka) and gly-gly (Wako Pure Chemical Industries, Osaka) were obtained commercially.

Diets. A 25% casein-sucrose purified diet which provides all the nutrients that meet the requirements of the rat (16) was used as the basal diet (Table 1). The addition of Am and GDF were made by replacing equal amounts of the basal diet to avoid altering nutrient proportion. Here, the control and test diets refer to the basal diet to which 5% Am was added and the basal diet to which 5% Am plus 5% GDF were added, respectively.

General treatment of animals. Male rats of the Wistar strain (Tokushima Jikken-Dobutsu Kenkyusho, Tokushima) were used in all experiments. After feeding the basal diet for 5–10 days for acclimatization, the animals were divided

![Fig. 1. Components of dietary fiber sample prepared from gobo, the roots of edible burdock (Arctium lappa L.). Pectin was determined by the method using hexametaphosphate (10). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid-resistant lignin were determined by the method of Van Soest (11,12). Hemicellulose and cellulose contents were calculated from the difference of NDF and ADF, and ADF and acid-resistant lignin (13).](image-url)
Table 1. Composition of basal diet.

| Ingredients                   | Amount (g/100 g of diet) |
|-------------------------------|--------------------------|
| Casein¹                       | 25.0                     |
| Corn oil²                     | 5.0                      |
| Mineral mixture³              | 4.0                      |
| Vitamin mixture⁴              | 1.0                      |
| Choline chloride⁵             | 0.2                      |
| Vitamin E granule⁶            | 0.05                     |
| Sucrose                       | —to make 100—            |

¹Lactic casein (30 mesh), purchased from New Zealand Dairy Board, Wellington.
²Just prior to preparing the diet, retinyl palmitate and ergocalciferol were mixed with corn oil to provide 4 mg and 15 μg, respectively, in kg diet. ³The mineral mixture contained (in g/kg): CaCO₃, 11.72; NaCl, 9.65; KH₂PO₄, 13.72; MgSO₄·7H₂O, 3.99; MnSO₄·H₂O, 0.37; Fe₃(C₆H₅O₇)·6H₂O, 0.25; CaHPO₄·2H₂O, 0.17; (in mg/kg diet) CuSO₄·5H₂O, 62.4; ZnCl₂, 56.8; Na₂MoO₄·2H₂O, 2.82; Co(CH₃COO)₂·4H₂O, 0.84; Na₂SeO₃, 0.59; KIO₃, 0.56. This mix was essentially the same as the mineral mixture-2 formulated by Ebihara et al. (14), with exception of following trace elements: Na₂SiO₃·9H₂O, Na₂B₄O₇·10H₂O, NiCl₂·6H₂O, NaF, Na₂HAsO₄·7H₂O, CrCl₃·6H₂O, SnSO₄·NH₄VO₃. ⁴This was identical with Harper’s mixture (15) with sucrose as a diluent. ⁵Added as a 50% solution in 50% ethanol. ⁶Trade name is “Juvera E granule,” purchased from Eisai Co., Tokyo. It contained 200 mg of all-rac-α-tocopheryl acetate per gram.

into groups on the basis of body weight and housed in individual suspended cages with screen-bottoms of stainless steel which were set in a room maintained at 23 ± 1°C and with a 12-h light (0800 to 2000) and 12-h dark (2000 to 0800) cycle.

Determination of jejunal sucrase activity. At the end of feeding periods, the small intestine was rapidly removed and 3 everted segments of 2 cm long were obtained from the second 15 cm of proximal jejunum, discarding the first 15 cm distal from the pylorus. Mucosal scrapings from one of the three segments were homogenized with a glass homogenizer in Krebs Ringer phosphate buffer (KRPB, pH 7.2), filled up to 25 ml and used for the determination of mucosal protein content and sucrase activity. Mucosal protein content was determined by the method of Lowry et al. (17), using bovine serum albumin as a standard.

Sucrase activity in homogenized jejunal mucosa was determined as follows: to 2.5 ml of prewarmed substrate solution (sucrose 116 mmol/liter KRPB), 2.5 ml out of 25 ml mucosal homogenate were added and the reaction mixture was stood for 10 min at 37°C with continuous shaking, then a flask containing the mixture was immersed into a boiling water bath to stop the reaction. After the mixture was centrifuged at 3,000 rpm for 10 min, the resultant supernatant was analyzed for glucose liberated from sucrose by the glucose oxidase method (18).

Sucrase activity in everted segment was also determined with 2 jejunal rings as
the enzyme source as described previously (19), which was our modification of the method of Ugolev et al. (20).

Response of jejunal sucrase to feeding Am with or without GDF in fed rats (experiment 1). Twenty-eight rats weighing about 67 g were divided into 4 groups of 7 rats after preliminary feeding of the basal diet for 5 days. Rats in group 1 were killed by decapitation before the beginning of experimental feeding to determine the initial activity of jejunal sucrase. The remaining 3 groups were given respective diets (the basal, control diet and test diets) *ad libitum* for 3 days, then animals were killed and everted segments of 2 cm long were made from the second 15 cm of the proximal jejunum. Sucrase activity and mucosal protein contents in the everted jejunal ring were determined in the manner described above.

Response of jejunal sucrase to feeding Am with or without GDF in fasted rats (experiment 2). After preliminary feeding for 10 days, 65 male rats weighing about 103 g were divided into 8 groups of 8 rats, then fasted for 3 days. Rats in groups 1 and 2 were killed before and after the fasting, respectively, and the remaining 48 rats were divided into 3 groups of 16 rats and fed the 3 kinds of diets used in experiment 1. On day 3 after refeeding, half of the animals in each group were killed and the remainder were killed after 14 days. After rapid removal of the small intestine, everted jejunal segments were prepared by the similar way described above and used for enzyme assay.

Digestion-absorption capacity of the jejunal segment perfused in situ with the medium containing sucrose and gly-gly after feeding Am with or without GDF (experiment 3). Rats weighing about 103 g were divided into 3 groups of 6 rats and given the 3 kinds of diets described above. In order to eliminate any possible effects of difference in body weight on digestion-absorption capacity, food intakes of rats fed the basal and test diets were adjusted to attain the same growth rate as that of rats fed *ad libitum* the control diet.

At the end of feeding period, jejunal perfusion was carried out in a similar manner described in the previous report (21): rats were anesthetized with intraperitoneal injection of a sodium pentobarbital solution (Nembutal, Abbott Laboratories, North Chicago, IL) and the abdomen was opened by a midline incision. Two small incisions were made on the antimesenteric borders of the proximal jejunum at 15 cm and 20 cm distal from the pylorus to produce a 5 cm segment. A silicon tubing with inner diameter of 0.5 mm was inserted into the proximal end of the segment and secured with ligature and the distal end was similarly connected with a tubing with an inner diameter of 3.0 mm. Blood supply to jejunal segment was maintained intact. The abdomen was temporarily closed.

After the segment was gently flushed twice with 5 ml of saline, the proximal tubing was connected with a syringe equipped on a perfusion pump (Compact Infusion Pump 979, Harvard Apparatus, MS. 02054). The segment was then perfused with 5 ml of KRPB containing sucrose (75 μmol) and gly-gly (150 μmol) at a calibrated rate of 35.7 μl/min, and the perfusate which flowed out from the distal end was collected. Concentrations of sucrose and dipeptide in the perfusate
were adopted from those in assay medium used by Ugolev et al. (20) for measuring activities of jejunal mucosal enzymes. At the end of perfusion period, the segment was again flushed with KRPB without substrates and the washing was combined with collected perfusate. After appropriate dilution, the mixture was analyzed for remaining sucrose and gly-gly. Sucrose was determined by the glucose oxidase method (18) after acid hydrolysis, and gly-gly was determined with an automatic amino acid analyzer (KLA-5, Hitachi, Ltd., Tokyo) without prior hydrolysis.

Statistical methods. All data were analyzed by analysis of variance (22), and by Duncan’s multiple range test (23), using a computer program developed by M. Mori and N. Kitahara, Department of Agricultural Chemistry, Hokkaido University. All statements of significant difference refer to 95% level of confidence.

RESULTS

Experiment 1

Table 2 shows that the feeding of control diet resulted in a remarkable growth retardation accompanied by significantly decreased food intake and food efficiency (g gain/g food intake), whereas feeding of the test diet supported growth equivalent to that of the basal diet-fed group. These distinct differences in growth responses were observed from the beginning of feeding. At the end of 3 days’ feeding, however, there were no decreases in sucrase activity and protein content of

Table 2. Changes in body weight, food intake, mucosal protein content and sucrase activity of the jejunum when experimental diets were fed for 3 days to non-fasted rats (experiment 1).

| Initial                                                                 | After feeding the following diets for 3 days |
|------------------------------------------------------------------------|---------------------------------------------|
|                                                                         | Basal (B) | B + 5%Am¹ | B + 5%Am + 5%GDF² |
| Body weight (g)            | 67.2 ± 1.8³ | 68.4 ± 2.4⁵ | 66.0 ± 2.7* | 82.4 ± 2.4⁵ |
| Food intake (g/3 days)     | —         | 33.7 ± 1.5* | 21.0 ± 1.8⁵ | 32.0 ± 0.9⁵ |
| Mucosal protein* (mg/2 cm jejunum) | 11.7 ± 0.35⁵ | 11.5 ± 0.43⁵ | 10.5 ± 0.61* | 12.6 ± 0.44⁵ |
| Sucrase activity* (µmol/2 cm jejunum) | 4.67 ± 0.42⁷ | 4.24 ± 0.34⁷ | 4.40 ± 0.20⁷ | 5.03 ± 0.48⁷ |

¹ Abbreviation of amaranth (Food Red No. 2); trisodium salt of 1-(4-sulfo-1-naphthylazo)-2-naphthol-3,6-disulfonic acid. ² Dietary fiber prepared from the roots of gobo, edible burdock (Arctium lappa L.). ³ Mean ± SEM (n = 7); values not sharing a common superscript letter within a line are significantly different (p < 0.05) as determined by Duncan’s multiple range test (23). ⁴ Mucosal protein of 2 cm jejunum which was obtained from second 15 cm segment from the pylorus. ⁵ Sucrase activity determined with everted jejunal ring was expressed as µmol of glucose produced when 2 cm segment of the everted jejunum was incubated with 5 ml medium containing 58 mmol/liter sucrose for 10 min.
Table 3. Change in body weight, food intake, mucosal protein content and sucrase activity of the jejunum when experimental diets were fed to rats fasted for 3 days (experiment 2).

|                          | Initial                      | After fasting for 3 days | After feeding fasted rats with the following diets for |     |     |
|--------------------------|-----------------------------|--------------------------|-------------------------------------------------------|-----|-----|
|                          |                             | Basal (B)                | B + 5%Am¹ | B + 5%Am + 5%GDF² | 3 days | 14 days |
|                          |                             |                          |           |                    |        |        |
| Body weight (g)          | 127 ± 3.5⁶,³                | 102 ± 3.5b               | 132 ± 2.0c | 102 ± 1.9b         | 129 ± 2.9a | 231 ± 5.7d | 147 ± 8.2 (7)⁷d | 217 ± 4.1e |
| Food intake (g/3 days)   | —                           | 40.7 ± 1.4a              | 24.1 ± 2.1b | 35.8 ± 1.6ab       |        |        |
| Mucosal protein³ (mg/2 cm jejunum) | 10.5 ± 0.48⁴d | 7.25 ± 0.36b | 12.1 ± 0.44c | 9.33 ± 0.38a | 11.7 ± 0.43⁴d | 17.1 ± 0.45e | 16.5 ± 0.65 (7)¹⁴ | 17.5 ± 0.63e |
| Sucrease activity⁶        | 3.94 ± 0.75⁵c               | 2.12 ± 0.20b           | 4.15 ± 0.20a | 3.21 ± 0.22c       | 3.81 ± 0.33²c | 4.69 ± 0.41⁴d | 5.49 ± 0.26 (7)²⁴ | 4.71 ± 0.27³d |

¹ Abbreviation of amaranth (Food Red No. 2); trisodium salt of 1-(4-sulfo-1-naphthylazo)-2-naphthol-3,6-disulfonic acid. ² Dietary fiber prepared from the roots of gobo, edible burdock (Arctium lappa L.). ³ Mean ± SEM (n = 8); values not sharing a common superscript letter within a line are significantly different (p < 0.05) as determined by Duncan's multiple range test (23). ⁴ The figure in parentheses indicates the number of rats which survived and on which data are based when this number was less than the original number per group. ⁵ Mucosal protein of 2 cm jejunum which was obtained from second 15 cm segment from the pylorus. ⁶ This means the activity of everted jejunal ring; details of the procedure are described in MATERIALS AND METHODS in this text.
segmental jejunal mucosa in the control rats as compared with values before the feeding or those in rats fed the basal diet.

**Experiment 2**

Table 3 shows results obtained when the same kinds of diet used in experiment 1 were fed to rats which had been fasted for 3 days. As the result of 3 days' fasting, rats had about 20% less body weight, 30% less mucosal protein and 50% less sucrase activity as compared with values before the fasting. When rats were fed the control diet, a remarkable delay in growth was again observed, especially at an early stage of feeding, and one of eight rats fed the control diet died on day 5. Rats fed the basal and test diets showed growth responses similar to those observed in experiment 1.

On the other hand, sucrase response to the control diet feeding in the fasted rats was different from that observed in fed rats. On day 3 after feeding the control diet, jejunal sucrase level was significantly lower than that in rats fed the basal diet or the test diet. After 14 days of feeding, however, the lowered activity in rats fed the control diet completely caught up to those in rats fed the latter two diets.

![Fig. 2](image)

**Fig. 2.** Time-course change in sucrase activity of jejunal mucosal homogenate from rats fasted for 3 days and then fed 3 different diets for up to 14 days (exp. 2). Diets are as follows: ⊙, fasted; ○, basal diet; ●, basal + 5% amaranth diet; △, basal + 5% amaranth + 5% dietary fiber from gobo (GDF), the roots of edible burdock (*Arctium lappa* L.). Vertical bars represent standard error of the mean of 8 rats. Values not sharing a common alphabetical letter differ significantly ($p < 0.05$) as determined by Duncan's multiple range test (23). These data indicate that 3 days' fasting itself decreased jejunal sucrase level to 50% of initial level, and that although amaranth-fed rats showed a delay in regaining the sucrase level at early stage of feeding, they had the same sucrase level as that of rats fed either the basal diet or the basal diet containing 5% amaranth plus 5% GDF after 14 days.
The same changes were also observed for mucosal protein content of the jejunum (Table 3); mucosal protein, as well as sucrase activity, in rats fed the test diet for 3 days was an intermediate value between those of rats fed the basal and control diets. Sucrase activity in homogenate of jejunal mucosa was also determined in experiment 2 (Fig. 2). Jejunal sucrase level determined by the activity in homogenized jejunal mucosa gave more clear-cut response to the fasting and subsequent feeding than that determined by the everted jejunal ring, although response of sucrase activity to fasting and feeding the three kinds of diets were essentially the same as that determined with everted jejunal ring. Although 3 days' fasting reduced the jejunal sucrase by 50% (Fig. 2), it showed a catch-up increase during subsequent feeding period even in rats fed the control diet. Sucrase level in control rats elevated with 3-day lag and attained the same level as that in rats fed the basal diet 14 days after feeding despite remarkable inter-group differences in body weight (Table 3). Rats fed the test diet regained the sucrase level at a significantly higher rate than that of rats fed the control diet during the initial 3 days, accompanied with increase in the mucosal protein content (Table 3).

**Experiment 3**

Table 4. Digestion-absorption capacity of the jejunal segment for sucrose and glycylglycine in rats fed 3 different diets by adjusting to attain the same growth rate for 14 days (as determined by in situ perfusion test, experiment 3)\(^1\)\(^2\).

| Diet                | Nutrient intake for 14 days (g) | Body weight (g) | Absorption of \(\mu\)mol substrate disappeared (5 cm jejunal segment) |
|---------------------|---------------------------------|-----------------|---------------------------------------------------------------|
| Basal diet          | 118 ± 1.2\(^a\)                | 127 ± 3.4\(^a\) | 29.3 ± 2.8\(^a\)                                             |
| Basal diet +5% amaranth\(^5\) | 151 ± 4.8\(^b\)                | 125 ± 4.8\(^a\) | 30.6 ± 4.3\(^a\)                                             |
| Basal diet +5% amaranth +5% GDF\(^6\) | 124 ± 3.8\(^a\)                | 128 ± 3.2\(^a\) | 30.4 ± 4.0\(^a\)                                             |

\(^1\)Initial body weight, 103 g (range, 96 to 110 g). \(^2\)Food intakes of rats fed the basal and basal +5% amaranth +5% GDF diets were adjusted to attain the same growth rate as that of rats fed the basal +5% amaranth diet ad libitum in order to eliminate any possible effects of difference in body weight on digestion-absorption capacity. \(^3\)Digestion-absorption capacity was evaluated from the difference in the concentrations of these substrates in the medium before and after perfusion. Details of the jejunal perfusion procedure are given in the text. \(^4\)Mean ± SEM (n = 7); values not sharing a common superscript letter within a column are significantly different (\(p < 0.05\)) as determined by Duncan's multiple range test (23). \(^5\)Amaranth (Food Red No. 2); trisodium salt of 1-(4-sulfo-1-naphthylazo)-2-naphthol-3,6-disulfonic acid, purchased from San-Ei Chemical Industries, Osaka. \(^6\)Dietary fiber prepared from the roots of gobo, edible burdock (Arctium lappa L.).
In experiment 3, we examined the effect of prolonged Am-feeding on digestion-absorption capacity of the jejunal mucosa. In order to eliminate any possible effect of difference in body weight, body weight gain of rats fed the basal and test diets was adjusted to that of rats fed ad libitum the control diet by restricting their daily nutrient intake. As a result, the basal and test diets supported the same growth rate as the control diet-fed rats with 22% and 18% less amounts of nutrients, respectively, than the latter diet (Table 4).

As shown in Table 4, absorption capacities for sucrose and gly-gly were not altered by feeding the control diet, indicating that digestion-absorption capacity or jejunal mucosal integrity was not affected even when fed 5% Am for 14 days.

**DISCUSSION**

In addition to Am, Kimura et al. (7, 24, 25) fed 10% polyethylene glycol 400, 10% Tween 60, 2% sodium taurocholate, 0.5% sodium deoxycholate and 2.5% laurylbenzene sulfonate to rats subjected to 2 days' fasting and observed lower activities in sucrase and alkaline phosphatase per unit length of jejunum than those in rats fed the basal diet for 4 days. From these results, they concluded that the reduction in segmental activity of jejunal enzymes should also result from exfoliation of the brush border membrane caused by these chemicals.

We examined the responses of the jejunal sucrase and glycyl-L-leucine hydrolase activities to feeding Am, and observed no changes in the activities after feeding the 4 or 5% level of Am for 4 h, 10 days and 21 days (19). Thus, our results were incompatible with those reported by Kimura et al. (7), but our experimental conditions were slightly different from theirs. The distinct differences from ours are in animal treatments: their rats were subjected to 2 days' fasting just before the experimental feeding.

Only when the Am-containing diet was fed to fasted rats for 3 days (experiment 2), we obtained the result similar to their observation that dietary Am resulted in a jejunal sucrase level significantly lower than that of rats fed the basal diet. However, 3 days' Am-feeding did not reduce a lowered sucrase level preformed in fasted rats (Table 3). These results suggest that the lowered sucrase activity found in rats fed Am for 3 days after 3 days' fasting resulted from a temporary delay of recovery from the fasting-induced malnutrition, but not due to release of membranous sucrase by Am-feeding.

Therefore, discrepancy between the results obtained by us (19) and by Kimura et al. (7) should be attributable to the differences in nutritional status of rats just before the experimental feeding, as judged by differences in sucrase level before Am-feeding between the fed and fasted rats (Tables 2 and 3). Indeed, McManus and Isselbacher (26) reported that the intestine of fed rats was heavier and the DNA content was greater than that of rats fasted overnight (15 to 16 h). Other investigators (27) have also shown that changes in cell population of the intestinal epithelium can be produced by fasting as well as by protein depletion.

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It seems likely that catch-up of jejunal sucrase lost by fasting depends on availability of nutrients in a refed diet, especially protein. In our previous studies (3), dietary Am was found to impair the digestibility and biological value of dietary casein. Furthermore, the lowered protein digestibility was associated with inhibitory effects of Am on digestion-absorption system, involving luminal digestion by trypsin, activities of membrane digestion for sucrose and dipeptides and active transport across the mucosal membrane of L-valine (19).

On the other hand, Tanaka and Okahara (28) reported that there were no differences in both the activity of leucine aminopeptidase and uptake of $\alpha$-aminoisobutyric-14C-acid by mucosal cells of the small intestine for all rats orally administered with Food Red No. 104, Food Yellow No. 5 and Acid Violet 6BN ($5 \times 10^{-3}$mmol/100g body weight) for 90 days unless activities were assayed in the presence of the food colors. These results also support the view that brush border membranes are maintained normal even after prolonged feeding of Am. In addition, results obtained from jejunal perfusion (experiment 3) well agreed with this view, because the ability of the perfused jejunal segment to absorb gly-gly and sucrose from perfusate was not altered even after 14 days of the Am-feeding (Table 4).

We have observed that when 0.5% Am solution was perfused through jejunal segment in situ, sucrase activity and DNA content in the effluent did not increase, whereas perfusion with 0.038% sodium deoxycholate increased release of sucrase and DNA (21). Furthermore, no morphological changes in jejunal brush border membranes were produced by 14 days' Am-feeding as judged with a scanning electron microscopy (29). From these and previous results, therefore, we conclude that although dietary Am decreases the availability of ingested nutrients, it does not impair the jejunal mucosal integrity. In fact, the adverse effect of amaranth immediately disappeared when it was removed from the diet (30).

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