EFFECT OF SEVERAL CHEMICALS ADDED TO A DIET ON INTESTINAL ENZYME ACTIVITIES OF RATS

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Summary The effects of anionic surface-active agents or of a laxative agent and dietary fiber added to a 20% casein high sucrose diet on changes in body weight, food consumption and gastrointestinal functions of rats were studied and compared with those of polyethyleneglycol 4000(PEG) and polyoxyethylene sorbitan monolaurate (Tween 20) reported in the previous study by Kimura, T., Furuta, H., Matsumoto, Y., and Yoshida, A. (*J. Nutr.*, **110**, 513(1980)). And the effects of these chemicals on intestinal enzyme activities, localized in the brush border membrane of the small intestine, *in vitro* were investigated. The effects of sodium laurylbenzenesulfonate(LBS) added to the diet on changes in the body weight, food consumption, feces and intestinal sucrase and alkaline phosphatase activities were more adverse than those of PEG or Tween 20, and the adverse effects of the former were not prevented with the concurrent feeding of Gobo dietary fiber(GDF) or soybean dietary fiber(Okara), although those of the latter two were prevented with the concurrent feeding of these dietary fibers. The massive addition of sodium stearate or sodium oleate to the diet did not produce any adverse effects on changes in the body weight, food consumption, feces and intestinal enzyme activities. The addition of magnesium sulfate, a laxative agent, to the diet induced diarrhea without significant changes in the body weight, food consumption and intestinal enzyme activities, and the induced diarrhea was not prevented with the concurrent feeding of GDF. Intestinal alkaline phosphatase activity *in vitro* was not affected by PEG, Tween 20, LBS or magnesium sulfate, but intestinal leucine aminopeptidase activity *in vitro* was inhibited by PEG, Tween 20, LBS or sodium oleate. Although intestinal sucrase and maltase activities were inhibited by LBS alone, the inhibition-effect of LBS on the sucrase activity was more severe than that of LBS on the maltase activity. From the experimental results, the mechanism by which these

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chemicals and dietary fiber exerted their effects on physiological functions was discussed.

Keywords small intestine, sucrase, maltase, leucine aminopeptidase, alkaline phosphatase, surface-active agent, laxative agent, dietary fiber

Ershoff *et al.* (1, 2) reported that adverse effects, significant growth retardation and severe diarrhea, of non-ionic surface-active agents added to a highly purified diet were counteracted by the concurrent feeding of dietary fiber, although the mechanism by which the chemicals and dietary fiber exerted their effects on the physiological functions remained unclear. From the recent findings that intestinal hydrolases within the brush border membrane of the villi were released *in vitro* and *in vivo* by several surface-active agents (3–5), we supposed the above-mentioned mechanism as follows. The added non-ionic surface-active agents to the diet exfoliated the brush border membrane and resulted in decreased absorption-capacities of dietary nutrients in the small intestine, and the resulting malabsorption induced diarrhea and reduction in food consumption, which was followed by significant growth retardation. And dietary fiber prevented the exfoliation of the brush border membrane due to the added chemicals and maintained at normal levels the absorption-capacities of the small intestine, fecal quality, food consumption, and growth. The segmental activities, that is, activities per length of the small intestine of intestinal sucrase, which localized in the brush border membrane in the villi (6), have been demonstrated to stay at a constant level regardless of the amount of diet consumed (7–10). In the previous study (10), to verify the hypothesis, the segmental sucrase activity was adopted as a criterion of the representative integrity and function of the brush border membrane, and the experimental results showed a great deal of evidence lending support to the hypothesis.

The present study was undertaken to find abundant evidence lending support to this hypothesis by the observation as follows; the relationships among changes in the body weight, food consumption, diarrhea, and hydrolase activities localized in the brush border membrane (11) of rats fed on diets containing anionic surface-active agents or a laxative agent and dietary fiber, and the effects of the chemicals on these enzyme activities *in vitro*.

EXPERIMENTAL

Animals and diets. Male rats of the Wistar strain² were individually housed in suspended wire-mesh cages in a room kept at 23–25°C with lighting automatically regulated to provide a 12-hr light period (08.00–20.00 hr) and a 12-hr dark period (20.00–08.00 hr). Composition of the basal diet in percentages: casein, 20; corn oil, 5; vitamin mixture (12), 2; salt mixture (13), 4; choline chloride, 0.2, and

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sucrose, 68.8. Retinyl palmitate, 350 IU, ergocalciferol, 35 IU, and all-*rac*- α -tocopherol, 5 mg, were added to 100 g of the diet. Tests were conducted with the following chemicals; sodium laurylbenzenesulfonate(LBS), polyoxyethylene sorbitan monolaurate (Tween 20), polyethylene glycol 4000(PEG), sodium stearate and sodium oleate, and magnesium sulfate as a laxative agent. The sources of dietary fiber used in the presence study were the root of edible burdock (*Arctium lappa*), "Gobo," a normal Japanese foodstuff, and soybean. The preparation and composition of Gobo dietary fiber(GDF) and soybean dietary fiber(Okara) have been described in previous studies (10, 14). The addition of chemicals, GDF and Okara to the diet was made without replacing any components of the diet.

Effects of chemicals and dietary fiber added to the diet on changes in body weight, food consumption, feces and intestinal enzyme activities. In experiment 1, rats after a 2-day fast were divided into 5 groups, and were refed *ad libitum* on the basal, 0.5% LBS, 1% LBS, 2.5% LBS or 5% LBS diet, respectively, for 3 days. In experiment 2, rats after a 2-day fast were divided into 3 groups and refed *ad libitum* on the basal, 2.5% LBS or 2.5% LBS plus 10% Okara diet for 4 days. They were killed in the morning on day 5 of the refeeding period, and the small intestine was rapidly removed, a jejunal segment representing the 15 cm segment distal to the pylorus being excised. The segment was slit longitudinally after rinsing with cold saline, and the mucosa was scraped with a piece of glass and homogenized with cold distilled water in a Polytron homogenizer (model PCO-2-110, Kinematica, Switzerland), and used for the determinations of protein content and enzyme activities. The experimental procedures in experiments 3 to 6 were carried out basically as per experiment 1, except for the experimental diet; in experiment 3, the basal, 2.5% LBS, 2.5% LBS plus 2.5% GDF, 2.5% LBS plus 5% GDF, and 2.5% LBS plus 10% GDF diet was used; in experiment 4, the basal and 10% sodium stearate diet; in experiment 5, the basal, 7.5% sodium oleate and 15% sodium oleate diet; in experiment 6, the basal, 0.5% magnesium sulfate, 1% magnesium sulfate, 2.5% magnesium sulfate, and 5% magnesium sulfate diet; in experiment 7, the basal, 5% magnesium sulfate and 5% magnesium sulfate plus 10% GDF diet, but in experiment 5 the protein content and enzyme activities of the mucosa were measured by the manner similar to that carried out in experiment 2, and in experiment 7 the jejunal segment of the small intestine was homogenized without scraping the mucosa and used for the determinations of the protein content and enzyme activities. In preliminary experiments there were no differences between the segmental enzyme activities determined with the homogenate of the mucosa and those determined with the homogenate of the small intestine. Throughout the experiments, changes in the body weight, food consumption and feces were observed during the refeeding period, and the determination of diarrhea was made by the observation of the apparent quality of feces.

Analytical procedures. Sucrase and maltase activities were determined by the method of Dahlqvist (15). Alkaline phosphatase activity was measured by the method of Kind and King (16). Leucine aminopeptidase activity was determined by

Table 1. Concentration of amaranth in lumen of small intestine in rats fed 0.5 or 1% amaranth diet.

Jejunum	Ileum
Relative concentration ¹	
7.0 ± 3.3 ²	53.3 ± 12.1

¹ This concentration is shown as the percentage to the concentration in the diet, as 100.

² Mean ± SE (*n* = 12),

the method of Goldberg and Rutenburg (17). The protein in the mucosa and the small intestine was determined by the method of Lowry *et al.* (18) using bovine serum albumin as a standard.

Effects of chemicals on intestinal enzyme activities in vitro. The enzyme preparation used was the homogenate, made by the same method as that in experiment 7, of the small intestine obtained from rats fed on the basal diet. The maximum concentration of the respective chemicals in the incubating medium for the above-mentioned determinations of intestinal enzyme activities corresponded with that in the lumen of the jejunum in rats fed on the diet containing a toxic dose of the chemicals. To determine the concentration of chemicals in the lumen of the small intestine, rats after one-day fasting were refed on a diet containing 0.5 or 1% amaranth, trisodium salt of 1-(4-sulfo-1-naphthylazo)-2-naphthyl-3,6-disulfonic acid, which was water-soluble, unabsorbable and easily detectable (19), for 2 days, and the concentration of amaranth in the lumen of the jejunum and ileum were colorimetrically assayed. The results are shown in Table 1, which demonstrated that dietary amaranth was diluted about 15-fold and 2-fold in the lumen of the jejunum and the ileum, respectively. From these findings 1% of Tween 20, PEG, sodium oleate and magnesium sulfate, and 0.1% of LBS respectively were adopted as the maximum concentration in the incubating medium.

Statistical analysis. Statistical analysis was done by the least significant difference calculated according to the method described by Snedecor and Cochran (20).

RESULTS

Effects of chemicals and dietary fiber added to the diet on changes in body weight, food consumption, feces and intestinal enzyme activities

The results of experiment 1 are shown in Table 2. The addition of LBS above the 1% level to the basal diet induced diarrhea, a remarkable reduction of gain in body weight and a significant decrease in food consumption. In experiment 2, as shown in Table 3, these effects of the addition of LBS at the 2.5% level to the basal diet were demonstrated as were those in experiment 1, and the weight of the intestinal segment of the 2.5% LBS group was significantly lower than that of the

Table 2. Effects of sodium laurylbenzenesulfonate (LBS) added to a 20% casein-high sucrose diet (HSD) on changes in body weight, food consumption and feces (Experiment 1).

Diet	HSD	HSD + 0.5% LBS	HSD + 1% LBS	HSD + 2.5% LBS	HSD + 5% LBS
Initial body wt. (g)	119 ± 7 ^{1,a}	125 ± 5 ^a	125 ± 4 ^a	129 ± 5 ^a	129 ± 5 ^a
Changes in body wt. (g/3 days)	25.5 ± 1.7 ^a	28.8 ± 2.3 ^a	14.5 ± 1.0 ^b	-0.1 ± 0.6 ^c	-10.3 ± 0.6 ^d
Food intake (g/day)	13.1 ± 0.9 ^a	13.0 ± 0.9 ^a	9.0 ± 0.3 ^b	6.0 ± 0.1 ^c	2.9 ± 1.0 ^d
Diarrhea	—	—	+	+	+

¹ Mean ± SE (n=4); means within line not sharing a common superscript letter differ significantly (p<0.05)

Table 3. Effects of LBS and soybean dietary fiber (Okara) added to HSD on changes in body weight, food consumption, feces and activities of intestinal enzymes (Experiment 2).

Diet	HSD	HSD + 2.5% LBS	HSD + 2.5% LBS, + 10% Okara
No. of rats	4	5	5
Initial body wt. (g)	143 ± 9 ¹	158 ± 9	156 ± 9
Change in body wt. (g/4 days)	26.0 ± 1.4	-3.6 ± 1.6 ^a	2.8 ± 0.8 ^{a, b}
Food intake (g/day)	15.4 ± 1.0	6.2 ± 1.1 ^a	7.1 ± 1.1 ^a
Diarrhea	—	+	+
Small intestine (mg/segment ²)	814 ± 54	664 ± 31 ^a	708 ± 18 ^a
Mucosal protein (mg/segment)	82.5 ± 8.1	68.5 ± 2.1	78.4 ± 1.8
Sucrase activity (U ³ /mg protein)	7.00 ± 0.81	3.33 ± 0.60 ^a	3.56 ± 0.22 ^a
(U ³ /segment)	561 ± 31	224 ± 33 ^a	277 ± 14 ^a
Alkaline phosphatase activity			
(U ⁴ /mg protein)	0.126 ± 0.037	0.052 ± 0.003	0.035 ± 0.006 ^a
(U ⁴ /segment)	12.4 ± 1.8	3.6 ± 0.2 ^a	2.8 ± 0.5 ^a

¹ Mean ± SE. ^a significantly different from HSD group (p<0.05), ^b significantly different from HSD+2.5%LBS group (p<0.05). ² Second 15 cm segment from the pylorus.

³ Micromoles of substrate split per hr. ⁴ Millimoles of substrate split per hr.

basal diet group. Sucrase and alkaline phosphatase activities either per mg of mucosal protein or per segment were significantly reduced by the feeding of the 2.5% LBS diet. These adverse effects of the 2.5% LBS diet were not prevented by the addition of Okara at the 10% level to this diet. The results of experiment 3 are represented in Table 4. As observed in the previous experiments the addition of

Table 4. Effects of LBS and Gobo dietary fiber (GDF) added to HSD on changes in body weight, food consumption and feces (Experiment 3).

Diet	HSD	HSD +2.5% LBS	HSD +2.5% LBS, +2.5% GDF	HSD +2.5% LBS, +5% GDF	HSD +2.5% LBS, +10% GDF
Initial body wt. (g)	66 ± 1.6 ^{1, a}	67 ± 1.2 ^a	66 ± 2.4 ^a	66 ± 0.8 ^a	67 ± 1.6 ^a
Change in body wt. (g/3 days)	16.7 ± 2.0 ^a	2.9 ± 0.3 ^b	5.8 ± 1.0 ^b	6.1 ± 0.3 ^b	5.4 ± 1.1 ^b
Food intake (g/day)	7.7 ± 1.8 ^a	3.6 ± 0.2 ^b	4.5 ± 0.3 ^b	4.8 ± 0.4 ^b	4.8 ± 0.3 ^b
Diarrhea	—	+	+	±	±

¹ Mean ± SE (*n* = 5); means within line not sharing a common superscript letter differ significantly (*p* < 0.05).

LBS at 2.5% level to the basal diet caused diarrhea and remarkable reduction in the body weight and food consumption, but the addition of GDF at 2.5, 5, and 10% levels to the 2.5% LBS diet did not prevent the adverse effects caused by the 2.5% LBS diet. On the other hand, in experiments 4 and 5 the massive addition of sodium stearate or sodium oleate to the basal diet did not affect changes in the body weight, food consumption, fecal quality, and intestinal enzyme activities (Tables 5 and 6). The addition of magnesium sulfate at 5% level to the diet induced diarrhea, but not significant reduction of gain in the body weight and food consumption as shown in Table 7 (experiment 6) and Table 8 (experiment 7). However, diarrhea induced by the feeding of 5% magnesium sulfate diet was not prevented by the addition of GDF at the 10% level to this diet (Table 8). There were no differences in intestinal enzyme activities between the basal diet group and the 5% magnesium sulfate group, but sucrase and leucine aminopeptidase activities in the 5% magnesium sulfate plus 10% GDF diet group were slightly but significantly higher than those in the basal diet group and the 5% magnesium sulfate group (Table 9).

Effects of chemicals on intestinal enzyme activities in vitro

In the preliminary experiments it was confirmed that these tested chemicals did not affect the determination of the respective product formed by the enzyme reactions mentioned above, except for that formed by the reaction of alkaline phosphatase in the incubating medium containing sodium oleate. Figures 1 to 5 represent the relationships between the concentration of PEG, Tween 20, LBS, sodium oleate, and magnesium sulfate in the incubating medium and sucrase, maltase, leucine aminopeptidase, and alkaline phosphatase activities. Sucrase, maltase, and alkaline phosphatase activities were affected by neither 0.5 nor 1% of

Table 5. Effects of sodium stearate added to HSD on changes in body weight, food consumption, feces and activities of intestinal enzymes (Experiment 4).

Diet	HSD	HSD + 10% Na stearate
Initial body wt. (g)	98 ± 1.7 ^{1, a}	99 ± 1.8 ^a
Final body wt. (g)	130 ± 2.8 ^a	132 ± 3.4 ^a
Food intake (g/day)	12.9 ± 0.6 ^a	14.1 ± 0.6 ^a
Diarrhea	—	—
Small intestine (mg/segment ²)	916 ± 8 ^a	913 ± 25 ^a
Mucosal protein (mg/segment)	80.0 ± 2.5 ^a	85.5 ± 4.8 ^a
Sucrase activity(U ³ /mg protein)	6.96 ± 0.33 ^a	6.22 ± 0.46 ^a
(U ³ /segment)	605 ± 53 ^a	535 ± 60 ^a
Alkaline phosphatase(U ⁴ /mg protein)	0.093 ± 0.008 ^a	0.101 ± 0.025 ^a
(U ⁴ /segment)	7.50 ± 0.73 ^a	8.73 ± 2.55 ^a

¹ Mean ± SE (n=5); means within line not sharing a common superscript letter differ significantly (p<0.05). ² Second 15 cm segment from the pylorus. ³ Micromoles of substrate split per hr. ⁴ Millimoles of substrate split per hr.

Table 6. Effects of sodium oleate added to HSD on changes in body weight, food consumption and feces (Experiment 5).

Diet	HSD	HSD + 7.5% Na oleate	HSD + 15% Na oleate
Initial body wt. (g)	108 ± 3 ^{1, a}	108 ± 2 ^a	106 ± 1 ^a
Change in body wt. (g/3 days)	20.2 ± 0.9 ^a	20.8 ± 1.3 ^a	20.0 ± 0.3 ^a
Food intake (g/day)	12.1 ± 0.3 ^a	11.9 ± 0.3 ^a	11.5 ± 0.3 ^a
Diarrhea	—	—	—

¹ Mean ± SE (n=5); means within line not sharing a common superscript letter differ significantly (p<0.05).

PEG, but leucine aminopeptidase activity was inhibited by 50% and 70% at 0.5% and 1% of concentration of PEG, respectively (Fig. 1). In the same degree as PEG, Tween 20 inhibited leucine aminopeptidase activity without affecting sucrase, maltase and alkaline phosphatase activities (Fig. 2). As shown in Fig. 3, without affecting alkaline phosphatase activity, LBS at a concentration of 0.1% completely inhibited sucrase and leucine aminopeptidase activities, and maltase activity was inhibited by 50%. Sodium oleate at a concentration of 0.5% completely inhibited leucine aminopeptidase activity, but at a concentration of 1% did not affect sucrase and maltase activities (Fig. 4). There were no significant inhibition-effects of

Table 7. Effects of magnesium sulfate added to HSD on changes in body weight, food consumption and feces (Experiment 6).

Diet	HSD	HSD +0.5% MgSO ₄	HSD +1% MgSO ₄	HSD +2.5% MgSO ₄	HSD +5% MgSO ₄
Initial body wt. (g)	66 ± 1.6 ^{1, a}	66 ± 1.6 ^a	66 ± 1.0 ^a	66 ± 2.5 ^a	67 ± 0.9 ^a
Change in body wt. (g/3 days)	16.7 ± 2.0 ^{ab}	18.0 ± 0.5 ^{ab}	21.1 ± 6.7 ^a	15.3 ± 1.3 ^{ab}	11.1 ± 1.7 ^b
Food intake (g/day)	7.7 ± 1.8 ^a	7.8 ± 0.2 ^a	7.8 ± 0.4 ^a	6.9 ± 0.3 ^a	6.3 ± 0.4 ^a
Diarrhea	—	—	—	±	+

¹ Mean ± SE (*n* = 5); means within line not sharing a common superscript letter differ significantly (*p* < 0.05).

Table 8. Effects of magnesium sulfate and GDF added to HSD on changes in body weight, food consumption, small intestine and feces (Experiment 7).

Diet	HSD	HSD +5% MgSO ₄	HSD +5% MgSO ₄ , +10% GDF
Initial body wt. (g)	99 ± 5.7 ^{1, a}	99 ± 2.4 ^a	99 ± 7.1 ^a
Change in body wt. (g/3 days)	20.2 ± 1.0 ^{ab}	18.8 ± 1.8 ^a	25.0 ± 2.6 ^b
Food intake (g/day)	10.5 ± 0.5 ^a	10.0 ± 0.4 ^a	11.2 ± 0.5 ^a
Small intestine (mg/segment ²)	676 ± 20 ^a	680 ± 14 ^a	693 ± 34 ^a
Intestinal protein (mg/segment)	87.0 ± 3.2 ^a	93.5 ± 2.7 ^a	92.4 ± 5.6 ^a
Diarrhea	—	+	±

¹ Mean ± SE (*n* = 5); means within line not sharing a common superscript letter differ significantly (*p* < 0.05). ² Second 15 cm segment from the pylorus.

magnesium sulfate, to the extent of a concentration of 1%, on sucrase, maltase, leucine aminopeptidase, and alkaline phosphatase activities (Fig. 5).

DISCUSSION

In the previous study (10) we investigated the adverse effects of PEG, Tween 20, or amaranth added to a 20% casein-high sucrose diet on growth, food consumption and gastrointestinal functions of rats, and the ameliorating effects of the concurrent feeding of dietary fiber. The addition of these chemicals to the diet induced severe diarrhea, a decrease in food consumption and remarkable reductions of gain in body weight and of segmental enzyme activities localized in

Table 9. Effects of magnesium sulfate and GDF added to HSD on activities of intestinal enzymes (Experiment 7).

Diet	HSD	HSD + 5% MgSO ₄	HSD + 5% MgSO ₄ , + 10% GDF
Sucrase activity(U ¹ /mg protein)	7.45 ± 0.53 ^{2, a}	7.52 ± 0.74 ^a	10.54 ± 1.05 ^b
(U ¹ /segment ³)	641 ± 25 ^a	707 ± 86 ^a	950 ± 42 ^b
Maltase activity(U ¹ /mg protein)	23.1 ± 1.61 ^a	21.8 ± 1.96 ^a	25.3 ± 1.14 ^a
(U ¹ /segment)	2,000 ± 150 ^a	2,030 ± 190 ^a	2,320 ± 140 ^a
Leucine aminopeptidase activity			
(U ¹ /mg protein)	2.93 ± 0.12 ^a	3.01 ± 0.10 ^a	3.39 ± 0.07 ^b
(U ¹ /segment)	255 ± 15 ^a	281 ± 11 ^{ab}	314 ± 20 ^b
Alkaline phosphatase activity			
(U ⁴ /mg protein)	0.101 ± 0.014 ^a	0.080 ± 0.009 ^a	0.088 ± 0.016 ^a
(U ⁴ /segment)	8.67 ± 1.04	7.59 ± 1.06 ^a	7.96 ± 1.41 ^a

¹ Micromoles of substrate split per hr. ² Mean ± SE (n=5); means within line not sharing a common superscript letter differ significantly (p<0.05). ³ Second 15 cm segment from the pylorus. ⁴ Millimoles of substrate split per hr.

Fig. 1. Effect of polyethyleneglycol 4000 (PEG) on activities of intestinal enzymes *in vitro*. O, sucrase; ●, maltase; x, leucine aminopeptidase; Δ, alkaline phosphatase.

the brush border membrane of the small intestine. The adverse effects of these chemicals added to the diet were completely prevented by the concurrent feeding of GDF or Okara. We supposed the mechanism of the effects of these chemicals and dietary fiber on physiological functions to be as follows. The chemicals added to the diet exfoliated the brush border membrane of the villi in the small intestine and resulted in decreased absorption-capacities of dietary nutrients and water in the small intestine. The resulting malabsorption induced diarrhea and reduction in food consumption, which was followed by significant growth retardation. The dietary fiber prevented the exfoliation of the brush border membrane due to the

Fig. 2. Effect of polyoxyethylene sorbitan monolaurate (Tween 20) on activities of intestinal enzymes *in vitro*. ○, sucrase; ●, maltase; ×, leucine aminopeptidase; △, alkaline phosphatase.

Fig. 3. Effect of sodium laurylbenzenesulfonate (LBS) on activities of intestinal enzyme activities *in vitro*. ○, sucrase; ●, maltase; ×, leucine aminopeptidase; △, alkaline phosphatase.

Fig. 4. Effect of sodium oleate on activities of intestinal enzyme activities *in vitro*. ○, sucrase; ●, maltase; ×, leucine aminopeptidase.

Fig. 5. Effect of magnesium sulfate on activities of intestinal enzymes *in vitro*. ○, sucrase; ●, maltase; ×, leucine aminopeptidase; △, alkaline phosphatase.

added chemicals, and maintained at normal levels the absorption-capacities of the small intestine, fecal quality, food consumption, and growth. In the previous study (10), changes in the segmental sucrase activity, adopted as a criterion of the representative integrity and function of the brush border membrane, in rats fed on the diets containing these chemicals appeared to parallel those in body weight, food consumption and fecal quality. These experimental results lent support to this hypothesis.

In the present study, the effects of LBS, an anionic surface-active agent, added to the diet on changes in body weight, food consumption, and fecal quality were more adverse than those of PEG or Tween 20 added to the diet. The adverse effects of PEG and Tween 20 appeared at 5 to 10% levels of the addition of these chemicals to the diet (10), whereas those of LBS appeared at 1 to 2.5% levels (Table 2). Furthermore, while the adverse effects of the addition of PEG or Tween 20 at the 10% level to the diet were completely prevented by the concurrent feeding of GDF or Okara at the 10% level in the diet containing these chemicals (10), those of LBS at the 2.5% level of the addition to the diet were unable to be prevented by the concurrent feeding of GDF or Okara even at the 10% level (Tables 3 and 4). However, the adverse effects of sodium stearate and sodium oleate, anionic surface-active agents (soaps), added to the diet at 10% and 15% levels respectively were not observed (Tables 5 and 6). The experimental results in the previous and present studies demonstrated that there were remarkable differences in these effects among these added surface-active agents.

In regard to the effects of these chemicals added to the diet on the activities of intestinal enzymes, localized in the brush border membrane of the small intestine (11), those of these chemicals on the segmental sucrase activity were considered to be more physiologically important; the segmental sucrase activity stayed at a constant level regardless of changes in the amount of the same diet consumed (7-10) and the induction-effect of the refed diet on the sucrase activity

was not affected by the addition of these chemicals into the refed diet.³ The addition of LBS as well as PEG or Tween 20 to the diet caused remarkable decreases in the segmental sucrase activity, but those caused by the addition of LBS to the diet were not prevented by the concurrent feeding of Okara (Table 3), although those caused by the addition of PEG or Tween 20 were completely prevented by the concurrent feeding of Okara (10). The addition of sodium stearate to the diet did not produce any decrease in the segmental sucrase activity (Table 5). These findings suggested that the LBS added exfoliated the brush border membrane more severe than the PEG or Tween 20 added, and that the added soaps did not affect the brush border membrane.

The results (Figs. 1 to 4) of the effects of PEG, Tween 20, LBS, or sodium oleate added at the maximum level to the incubating medium for the determinations of intestinal enzyme activities demonstrated that alkaline phosphatase activity was not affected by the addition of PEG, Tween 20, or LBS, and that leucine aminopeptidase activity was almost entirely inhibited by the respective addition of PEG, Tween 20, LBS, and sodium oleate, whereas the addition of LBS partially inhibited maltase activity and completely inhibited sucrase activity. From these findings and the experimental results of the effects of these chemicals added to the diet on the intestinal enzyme activities, the effects of these chemicals on maltase and sucrase activities were found to be physiologically more important than those on alkaline phosphatase and leucine aminopeptidase activities. In spite of the fact that the addition of PEG or Tween 20 to the incubating medium did not affect sucrase activity, decreases in the segmental sucrase activities of rats fed on the diets containing PEG or Tween 20 strongly suggested that PEG and Tween 20 exfoliated the brush border membrane. And the adverse effects of LBS added to the diet were considered to occur by the direct inhibition-effect of LBS on sucrase and maltase activities together with the exfoliation of the brush border membrane caused by LBS.

The addition of magnesium sulfate at the 5% level to the diet clearly induced diarrhea, which was not prevented by the concurrent feeding of GDF at 10% level in the diet containing magnesium sulfate, and the addition of magnesium sulfate to the diet and the concurrent feeding of GDF did not bring about any changes in the food consumption (Tables 7 and 8). Furthermore, the addition of magnesium sulfate to the diet did not cause any decreases in sucrase, maltase, leucine aminopeptidase, and alkaline phosphatase activities in the small intestine (Table 9), and these enzyme activities were not substantially affected by the existence of magnesium sulfate in the incubating medium. These findings demonstrated that diarrhea induced by the addition of magnesium sulfate to the diet was definitely different from diarrhea induced by PEG, Tween 20, or LBS. Induction of the former involved the osmotic effect of magnesium sulfate in the lumen of the small intestine, which was the main absorption-site of water as well as of dietary

³ Unpublished observation.

nutrients(21), whereas that of the latter involved the decreased absorption-capacities of water and nutrients in the small intestine. Increases in the intestinal enzyme activities without preventing diarrhea caused by the addition of GDF to the diet containing magnesium sulfate (Table 9) might be a counteraction of the small intestine due to GDF against the osmotic effect of magnesium sulfate in the lumen.

These finding obtained in the present study and the previous study(10), together with the fact that GDF itself did not have adsorptive capacities for these chemicals,⁴ lent strong support to the above-mentioned hypothesis.

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