Mechanisms of Toxicities of Some Detergents Added to a Diet and of the Ameliorating Effect of Dietary Fiber in the Rat

Toshizo Kimura,* Hitomi Imamura, Kazutomi Hasegawa, and Akira Yoshida

Laboratory of Nutritional Biochemistry, Department of Agricultural Chemistry, Nagoya University, Chikusa-ku, Nagoya 464, Japan

(Received November 30, 1981)

Summary Mechanisms of the adverse effects of dietary Tween 20, Tween 60, Span 20, sodium taurocholate (NaTC), sodium deoxycholate (DOC), sodium laurylbenzene sulfonate (LBS) and sodium dodecyl sulfate (SDS), and of the ameliorating effect of the concurrent feeding of dietary fiber were investigated along with releases of the hydrolase activities, which were localized in the brush border membrane of rat small intestine, during a jejunum perfusion in vivo. The releases of sucrase, maltase and alkaline phosphatase activities from the jejunum with Ringer bicarbonate solution (RBS) perfusion for 150 min proceeded at a constant rate after RBS perfusion for the first 30 min. The detergents were perfused after RBS perfusion for 60 min. In Tween 20- or 60-RBS perfusion at the 2% level, the released sucrase activity gradually increased, reaching a level 3 times that with RBS perfusion 90 min after the beginning of Tween 20- or Tween 60-RBS perfusion. With NaTC- or DOC-RBS perfusion at the 0.5% or 0.2% level respectively, the released sucrase activity reached a level 3 to 4 times that with RBS perfusion within 30 min of the beginning of NaTC- or DOC-RBS perfusion, but that with Span 20-RBS perfusion at the 2% level was slightly lower compared with that with RBS perfusion. On the other hand, with SDS- or LBS-RBS perfusion at the 0.5% level, the released alkaline phosphatase activity rapidly reached a level 3 to 4 times as high as that with RBS perfusion. The inclusion of Gobo dietary fiber at the 0.04% level with Tween 20-RBS perfusion completely eliminated the releasing effect of Tween 20 on sucrase activity. These results suggest that the primary cause of the adverse effects of the feeding of these detergents is the exfoliating or releasing effect thereof on the brush border membrane, together with the inhibitory effect of some of these detergents on intestinal

* Present address: Laboratory of Food and Nutrition, Department of Home Economics, Osaka Kyoiku University Ikeda, Johnan, Ikeda, Osaka 563, Japan.
disaccharidase activities, and that the dietary fiber prevents the exfoliating or releasing effects of several detergents on the brush border membrane. **Key Words** small intestine, sucrase, maltase, alkaline phosphatase, perfusion, detergent, dietary fiber

The role of dietary fiber in counteracting the toxic effects, significant growth retardation, and severe diarrhea caused by some non-ionic detergents added to the diet has been described by Ershoff et al. (1, 2), but the mechanisms by which these chemicals and dietary fiber exert their effects on the physiological functions remain unclear. We recently supposed the mechanisms by which these chemicals and dietary fiber affect physiological functions to be as follows: the chemicals added to the diet exfoliate or release the brush border membrane of the villi in the small intestine, resulting in decreased absorption capacities for dietary nutrients and water in the small intestine. The resulting malabsorption induces diarrhea and a reduction in food consumption, followed by significant growth retardation. The dietary fiber prevents the exfoliation or release of the brush border membrane due to the chemicals added to the diet and maintains at a normal level the absorption capacities of dietary nutrients and water in the small intestine, fecal consistency, food consumption and growth.

In the previous studies (3–5) undertaken to verify this hypothesis, the segmental sucrase activity of the jejunum was adopted as a criterion of the representative integrity and function of the brush border membrane, and the experimental results produced a great deal of evidence lending support to the hypothesis. The present study was undertaken to find confirmaible evidence lending support to the hypothesis by the observation of the following: the release of the hydrolase activities localized in the brush border membrane of the rat jejunum (6), during a jejunum perfusion in vivo using a Ringer bicarbonate solution containing one of several detergents and dietary fiber.

**EXPERIMENTAL**

Male rats of the Wistar strain (Shizudokyo, Shizuoka, Japan) weighing approximately 120g were used. Tests were conducted with the following chemicals; polyoxyethylene sorbitan monolaurate (Tween 20), polyoxyethylene sorbitan monostearate (Tween 60), sorbitan monolaurate (Span 20), sodium taurocholate (NaTC), sodium deoxycholate (DOC), sodium dodecyl sulfate (SDS) and sodium laurylbenzene sulfonate (LBS). The source of dietary fiber used in the present study was the root of the edible burdock (Arctium lappa), “Gobo,” a common Japanese foodstuff. The preparation and composition of Gobo dietary fiber (GDF) have been described in previous studies (3, 7). The animals fasted with access to water overnight were anesthetized with pentobarbital sodium (5 mg/100 g body weight). A proximal jejunal segment (about 10 cm in length) was perfused with Ringer
Table 1. Releases of hydrolase activities localized in brush border membrane of rat jejunum during jejunum perfusion in vivo with Ringer bicarbonate solution.

| Min after beginning of perfusion | 0 to 30 | 30 to 60 | 60 to 90 | 90 to 120 | 120 to 150 |
|----------------------------------|--------|---------|---------|-----------|-----------|
| Protein                          |        |         |         |           |           |
| mg                               | 5.89 ± 2.05 | 2.43 ± 0.56 | 2.24 ± 0.48 | 2.59 ± 0.59 | 2.70 ± 0.46 |
| Relative amount                  | 233 ± 56 | 100     | 92 ± 6  | 108 ± 15  | 117 ± 12  |
| U                                | 30.4 ± 2.6 | 3.78 ± 0.59 | 3.13 ± 0.62 | 3.96 ± 0.66 | 2.82 ± 0.82 |
| Relative activity                | 883 ± 113 | 100     | 89 ± 21 | 101 ± 18  | 81 ± 18   |
| Maltrate activity                | 98.8 ± 13.8 | 17.3 ± 2.5 | 13.4 ± 2.9 | 16.5 ± 4.7 | 15.1 ± 4.8 |
| Relative activity                | 582 ± 51  | 100     | 76 ± 16 | 86 ± 21   | 88 ± 26   |
| Alkaline phosphatase activity    | 458.6 ± 97.3 | 31.5 ± 6.1 | 24.0 ± 5.9 | 25.6 ± 6.1 | 27.2 ± 5.8 |
| Relative activity                | 1,472 ± 382 | 100    | 81 ± 12 | 84 ± 9    | 105 ± 13  |

*Mean ± SE (n=6). b The relative amount and activity represent the ratios to those released with Ringer bicarbonate solution from the 30th to 60th min after the beginning of perfusion in each of the jejunum segments used, as 100. c Micromoles of substrate split per hour.

bicarbonate solution (RBS) (8) at a constant rate (0.5 ml/min). Perfusion was continued for 150 min, allowing 30 min for equilibration and 120 min for collection of the perfusate in 30 min aliquots. Table 1 shows changes in the releases of enzyme activities and protein from the jejunum into the perfusate. These releases proceeded at a constant rate after the first 30 min of RBS perfusion. The constant liberation of the enzymes corresponded to physiological cell desquamation (9). Expressing the data as the relative activities of these enzymes, which were represented as ratios (these relative activities to those released in the second 30 min RBS perfusion in each of the jejunum segments used), removed the possibility of differences in physiological condition among rats, in length of jejunum used, and in flow-rate of perfusion among experiments. The relative activities of enzymes released with RBS perfusion proceeded substantially at a constant rate for 120 min after the first 30 min RBS perfusion. To investigate the effect of detergents and dietary fiber on releases of these enzyme activities, rat jejunum in experimental groups was perfused with RBS for 60 min, followed by RBS containing one of the detergents and dietary fiber for 90 min, and the activities of these released enzymes were compared with those in the second 30 min RBS perfusion, values of relative activity being obtained. Concentrations of detergents added to RBS were one-third to one-fifth their toxic levels, except for Span 20 added to a high sucrose diet (3), since these chemicals in the diet were diluted by one-half to one-tenth in the lumen of the small intestine with drinking water and secretion water (4). The sucrase activity released with these RBS
perfusions except for SDS- and LBS-RBS perfusion was assayed, but in SDS- and LBS-RBS perfusion the alkaline phosphatase activity was measured, because SDS and LBS inhibited intestinal disaccharidase activities in vitro (4). Sucrase and maltase activities were assayed according to the method of Dahlqvist (10). Alkaline phosphatase activity was determined by the method of Kind and King (11). Protein determination was done by the method of Lowry et al. (12) using bovine serum albumin as a standard. Statistical analysis was done by Student's "t" test used to determine significant differences between treatment means (13).

RESULTS AND DISCUSSION

As shown in Fig. 1, the release of sucrase in Tween 20- or Tween 60-RBS perfusion after the first 30 min of perfusion was higher than that with RBS perfusion, and increased about 3 times that with RBS perfusion 90 min after the beginning of perfusion. The release of sucrase activity with Span 20-RBS perfusion

![Fig. 1. Releases of sucrase activity from rat jejunum with time. The jejunum was perfused with Ringer bicarbonate solution (RBS) for 60 min, followed by RBS or RBS-containing detergents and dietary fiber for 90 min. The relative activity of sucrase is shown as the ratio to the activity released with RBS perfusion during the second 30 min period, as 100. Vertical bars represent the SE of the mean for six rats. *p<0.05 versus RBS perfusion. ●, RBS perfusion; ○, RBS perfusion with 2% Tween 20; □, RBS perfusion with 2% Tween 60; ●, RBS perfusion with 2% Tween 20 and 0.04% GDF; △, RBS perfusion with 0.2% sodium deoxycholate; ▲, RBS perfusion with 0.5% sodium taurocholate; ×, RBS perfusion with 2% Span 20.](image-url)
Fig. 2. Releases of alkaline phosphatase activity from rat jejunum with time. The jejunum was perfused with Ringer bicarbonate solution (RBS) for 60 min, followed by RBS or RBS-containing detergents for 90 min. The relative activity of alkaline phosphatase is shown as the ratio to the activity released with RBS perfusion during the second 30 min period, as 100. Vertical bars represent the SE of the mean for six rats. * $p<0.05$ versus RBS perfusion. ○, RBS perfusion; ○, RBS perfusion with 2% Tween 20; △, RBS perfusion with 0.5% sodium dodecyl sulfate; ×, RBS perfusion with 0.5% sodium laurylbenzene sulfonate.

was slightly lower than that with RBS perfusion. The presence of NaTC or DOC at the 0.5% or 0.2% level respectively in RBS increased greatly the release of sucrase, which was 3 to 6 times as high as that with RBS perfusion during all the periods used. The release changes in maltase and alkaline phosphatase activities and protein in Tween 20-, Tween 60- or NaTC-RBS perfusion were similar to those in sucrase activity, and the ratios of these released enzymes to the released protein in these perfusions were similar to those with RBS perfusion. This similarity suggested that Tween 20, Tween 60 and NaTC acceleratedly exfoliated or released the brush border membrane of the small intestine. On the other hand, these ratios with DOC-RBS perfusion were three times as high as those with the other perfusion. The effect of DOC suggested to be the solubilization of the epithelial cell rather than the exfoliation of the membrane.

As shown in Fig. 2, the presence of LBS at the 0.5% level in RBS produced a significantly higher release of alkaline phosphatase compared with that due to the
presence of the other chemicals in RBS. However, the RBS perfusion containing SDS at the 0.5% level greatly increased the release of alkaline phosphatase activity, which was 3 to 6 times as high as that in RBS perfusion during all the periods used. The release of alkaline phosphatase activity in RBS perfusion using Tween 20 at the 2% level was not as remarkable as with LBS- or SDS-RBS perfusion after the second 30 min perfusion. The ratio of the released alkaline phosphatase to the released protein in Tween 20- or SDS-RBS perfusion was similar to that in RBS perfusion. However, the ratio in LBS-RBS perfusion was three times as high as that in the other perfusion. The effects of LBS as well as DOC were suggested to be the solubilization of the epithelial cell rather than the exfoliation of the membrane.

These results indicated that there were significant differences in the exfoliating or releasing effect of these detergents on the brush border membrane of the small intestine. NaTC, DOC, SDS and LBS had a high exfoliating or releasing effect on the brush border membrane, Tween 20 and 60 had a low effect, and Span 20 did not have any effect. The differences in the adverse effects caused by the feeding of these detergent corresponded with those in the exfoliating or releasing effect of these detergents on the brush border membrane. The adverse effect of Tween 20 or 60 feeding was produced by the presence of a massive content, at the 10% level, in the diet, being prevented by the concurrent feeding of a small amount of GDF (3, 5). On the other hand, the effect of DOC, NaTC, SDS or LBS feeding was produced by the presence of a small amount, at the 0.5 or 2% level, in the diet, and was more deteriorative than that of massive Tween 20 or Tween 60 feeding. The deteriorative effects of NaTC, DOC, SDS or LBS were not ameliorated by the concurrent feeding of a massive amount of GDF (4, 5). However, the adverse effect of massive Span 20 feeding, which was not ameliorated with the concurrent feeding of dietary fiber (1), was considered to be different from that of Tween 20 or 60 feeding. From the results obtained using the perfusion technique, it was suggested that the primary cause of the adverse effect of Span 20 feeding would be some factors other than the exfoliating or releasing effect of detergents on the brush border membrane.

In the previous study (3), the adverse effect of Tween 20 added to the high sucrose diet at the 10% level on growth, food consumption, fecal consistency and intestinal sucrase activity was completely prevented by the concurrent feeding of GDF at the 2.5% level. Furthermore, in a preliminary experiment in the present study, even the concurrent feeding of GDF at the 0.5% level in the 10% Tween 20 diet showed a significant ameliorative effect. These findings strongly suggested that if the exfoliation of the brush border membrane due to Tween 20 was a primary component of the adverse effect of dietary Tween 20, the significant release of sucrase with RBS perfusion containing Tween 20 is expected to be preventable by the concurrent presence of a small amount of GDF in the perfusion medium. As shown in Fig. 1, the sucrase activity in the RBS perfusion containing Tween 20 and GDF at 2% and 0.04% levels respectively was lower than that with the RBS containing Tween 20 alone. The presence of GDF in the perfusion medium clearly indicated presence of a protective effect of GDF on the exfoliation of the brush
INTESTINAL ENZYMES AND CHEMICALS

These experimental results supported the suggestion that the primary cause of the adverse effects in rats fed on the diet containing these detergents was the exfoliating or releasing effect thereof on the brush border membrane, together with the inhibitory effect of some of these detergents on intestinal disaccharidase activities (4, 14), and that the ameliorating effect of GDF on the adverse effects of Tween 20 and Tween 60 was produced by the physicochemical affinity of GDF with the brush border membrane, which exceeded the exfoliating or releasing effect of these detergents.

This study was supported in part by a Grant No. 56560082 from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1) Ershoff, B. H. (1960): Beneficial effects of alfalfa meal and other bulk-containing or bulk-forming materials on the toxicity of non-ionic surface-active agents in the rats. J. Nutr., 70, 484-490.
2) Ershoff, B. H., and Marshall, W. E. (1975): Protective effects of dietary fiber in rats fed toxic doses of sodium cyclamate and polyoxyethylene sorbitan monostearate (Tween 60). J. Food Sci., 40, 357-361.
3) Kimura, T., Furuta, H., Matsumoto, Y., and Yoshida, A. (1980): Ameliorating effect of dietary fiber on toxicities of chemicals added to a diet in the rat. J. Nutr., 110, 513-521.
4) Kimura, T., Iwata, E., Watanabe, K., and Yoshida, A. (1980): Effect of several chemicals added to a diet on intestinal enzyme activities of rats. J. Nutr. Sci. Vitaminol., 26, 483-496.
5) Kimura, T., Iwata, E., Watanabe, K., and Yoshida, A. (1981): Effect of several detergents and dietary fiber added to a diet on intestinal sucrase activity in rats. J. Nutr. Sci. Vitaminol., 27, 389-392.
6) Eichholz, H. (1969): Fractions of the brush border. Fed. Proc., 28, 30-34.
7) Takeda, H., and Kiriyama, S. (1979): Correlation between the physical properties of dietary fibers and their protective activity against amaranth toxicity in rat. J. Nutr., 109, 388-396.
8) Laser, H. (1961): in Biochemist' Handbook, ed. by Long, C., Richard Clay & Co., Ltd., Bungay Suffolk, pp. 58-60.
9) Sigdestad, C. P., Hagemann, R. F., and Lesher, S. (1970): New method for measuring intestinal cell transit time. Gastroenterology, 58, 47-48.
10) Dahlqvist, A. (1964): Method for assay of intestinal disaccharidase. Anal. Biochem., 7, 18-25.
11) Kind, P. R. N., and King, E. J. (1954): Estimation of plasma phosphate by determination of hydrolyzed phenol with aminoantipyrine. J. Clin. Pathol., 7, 322-326.
12) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951): Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193, 265-275.
13) Snedecor, G. W., and Cochran, W. G. (1967): Statistical Methods (Japanese Ed.), Iowa State Univ. Press, Ames, Iowa, pp. 246-263.
14) Kimura, T., Watanabe, K., Iwata, E., and Yoshida, A. (1981): Nutritional significance of intestinal sucrase activity in rats. J. Nutr. Sci. Vitaminol., 27, 485-488.

Vol. 28, No. 5, 1982