Jpn. J. Cancer Res. 86, 523–529, June 1995

Effects of Apple Pectin on Fecal Bacterial Enzymes in Azoxymethane-induced Rat Colon Carcinogenesis

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Because of the potential significance of colonic bacteria in colon carcinogenesis, we investigated the effect of pectin of different types on fecal bacterial enzymes (β-glucuronidase, β-glucosidase and tryptophanase) at various periods of time after feeding rats with pectin-containing diets during azoxymethane-induced colon carcinogenesis. The diet supplemented with 20% apple pectin or 20% citrus pectin decreased the multiplicity of colon tumors, and the number of tumors was significantly decreased in the group fed apple pectin. The incidence of colon tumors in the apple pectin group was lower than that in the control group. The mean tumor size was similar among the three groups. Apple pectin feeding decreased fecal β-glucosidase and tryptophanase levels. Furthermore, a significant decrease in the activity of β-glucuronidase was observed in the apple pectin group during the initiation phase. These findings suggest that the protective effect of pectin on colon carcinogenesis may be dependent on the type of pectin and be related to the decrease of β-glucuronidase activity in the initiation stage of carcinogenesis.

Key words: Dietary fiber — Colon cancer prevention — Apple pectin — Azoxymethane — Fecal bacterial enzymes

Epidemiological data strongly suggest the importance of dietary factors in the etiology of colon cancer. A protective effect of dietary fiber and fiber-containing foods has been suggested.[1)] For chemically induced colon cancer in animals, the kind of dietary fiber may influence the number of colon tumors. As regards pectin, there are contradictory reports as to whether or not it is effective in experimental colon carcinogenesis.[2–9)] Most of the previous investigators used citrus pectin. However, we found that apple pectin inhibited azoxymethane (AOM)-induced colon carcinogenesis in rats.[10,11)] Apple pectin has stronger bacteriostatic action on *Staphylococcus aureus, Streptococcus faecalis, Pseudomonas aeruginosa* and *Escherichia coli* in comparison with citrus pectin.[12)] Therefore, apple pectin may more strongly influence the intestinal microflora and bacterial enzyme activities. The intestinal bacteria may play a significant role in the pathogenesis of colon cancer,[13)] because their enzymes are important in the metabolism of procarcinogens and production of tumor promoters in the colon.[13–16)]

No report has appeared concerning the effect of apple pectin on fecal bacterial enzymes in relation to experimental colon carcinogenesis. The purpose of the present study was to investigate the effects of two kinds of pectin, apple pectin and citrus pectin, on fecal bacterial β-glucuronidase, β-glucosidase and tryptophanase activities at various periods of time during AOM-induced colon carcinogenesis in rats.

MATERIALS AND METHODS

Animals and diets Male Donryu rats from Shizuoka Laboratory Animal Center (Shizuoka), 4 weeks old, each weighing about 200 g were used and placed in wire-mesh cages to prevent coprophagia. Water was administered *ad libitum* during the experiments. The room was air-conditioned at 22–23°C with a relative humidity of 50 to 60%. The light was switched on at 7 a.m. and off at 7 p.m. A total of 62 rats were divided into 3 groups; the control group fed a basal diet, MM-3 (Table I, purchased from Funabashi Farms, Chiba), the citrus pectin (CP) group fed the basal diet containing 20% CP (USA-SAG type, DD slow set, The Copenhagen Pectin Factory Ltd., Copenhagen, Denmark), and the apple pectin (AP) group fed the basal diet containing 20% AP (OM type, Herbstreith & Fox, Neuenburg, Germany). Body weights were recorded once a week, and food intake and excreted fecal weight in 24 h were measured for each dietary group at 9 weeks after the first AOM injection.

Carcinogenesis From 2 weeks after starting diet administration, animals (6 weeks old) were given subcutaneous injections of AOM (7.4 mg/kg) (Sigma Chemical Company, St. Louis, USA) once weekly for 10 weeks. Rats were killed 30 weeks after the first injection of AOM.

Histopathological examination Complete necropsies were performed for all animals. Grossly visible colonic tumors were recorded and tabulated. Tumor size was

estimated by using the following equation: Tumor size = $a \times b$, where a and b are the length and width (mm), respectively. All tissues were fixed in 10% buffered formalin, processed by the conventional methods, and stained with hematoxylin and eosin for histological diagnosis.

Fecal moisture Fresh fecal pellets were collected for fecal moisture determination 2 weeks after starting diet administration (before AOM injection; week 0), and at the 11th, 19th and 30th weeks after the first AOM injection. It was determined by weighing a portion of freshly collected feces before drying (wet weight) and after drying at 100°C for 4 h (dry weight). Then,

$$\text{Fecal moisture } (\%) = \frac{\text{wet weight (g)} - \text{dry weight (g)}}{\text{wet weight (g)}} \times 100$$

Fecal bacterial enzyme activities Fresh fecal pellets were also collected for assay of the following enzyme activities at the same time as the collection for fecal moisture determination.

Table I. Percentage Composition of Basal Diet, MM-3[a)]

Ingredient	Percentage
Crude carbohydrate[b)]	54.5
Crude protein[c)]	20.1
Crude fat[d)]	4.4
Ash	8.8
Crude cellulose[e)]	5.2
Water	7.0

a) 409.0 cal/100 g; Ca 1.66%, P 1.24%; contents of vitamins, A, D_3, E, B_1, B_2, and B_6 are 10 and 2 IU, and 50, 10, 10 and 10 μg/g diet.
b) The main sources of crude carbohydrate are corn, wheat and rice-bran.
c) The main sources of crude protein are fish meal and soybean cake.
d) The main sources of crude fat are corn oil and lard.
e) No special material was added as a source of crude cellulose.

β-Glucosidase assay mixture contained 3.0 μmol of substrate, p-nitrophenyl-β-D-glucopyranoside, 50 mM potassium phosphate buffer (pH 7.2), and a suitable amount of fecal suspension in a final volume of 1.0 ml. The mixture was incubated at 37°C for 15 min and the reaction was stopped by the addition of 0.25 ml of 5% Na_2CO_3. The enzyme activity was determined by spectrophotometrically measuring the absorption of produced p-nitrophenol at 405 nm. β-Glucuronidase activity was determined in the same way as β-glucosidase assay except that the substrate was p-nitrophenyl-β-D-glucuronide.[17] Tryptophanase activity was determined by the method of Demoss and Moser.[18]

Statistical analysis The significance of differences between the control and experimental groups was tested by using the χ^2 test and analysis of variance. A difference was considered statistically significant when the P value was 0.05 or less.

RESULTS

General observations Table II shows the body weights, daily food consumption and fecal weights. Animals fed the pectin diet had significantly lower body weights than did the animals fed the control diet ($P<0.001$). Animals fed the pectin diet consumed approximately the same amounts of food as those fed the control diet. The fecal weight was significantly increased in animals fed the apple pectin compared with those fed the control diet ($P<0.05$).

Tumor induction Table III summarizes the number and incidence of colon tumors. The incidences of total colon tumors in the control group and CP group were 83.3% (15/18) and 55.0% (11/20) ($P<0.1$), respectively, and the incidence of 37.5% in the AP group (9/24) was significantly lower than that in the control group ($P<0.01$). Furthermore, the incidence of carcinomas in the AP group (29.2%) was also significantly lower than that in the control group ($P<0.001$). The average numbers of

Table II. Effect of Pectin on Rat Body Weight, Food Consumption and Fecal Weight

	Body weight (g)				Food consumption[c)] (g/day)	Fecal weight[c)] (g/day)
	0[a)]	10	20	30 week[b)]		
Control	209.5±2.0[d)]	489.3±8.6	606.2±9.8	648.1±13.0	24.0±1.4	7.1±0.7
Citrus pectin	200.3±2.1	436.3±5.4[e)]	528.9±5.9[e)]	560.8±7.3[e)]	20.5±1.4	6.4±0.3
Apple pectin	202.0±2.3	429.7±9.7[e)]	510.7±10.1[e)]	554.4±12.9[e)]	24.2±0.6	10.5±0.8[f)]

a) Two weeks after starting diet administration.
b) Weeks after the first AOM injection.
c) Nine weeks after the first AOM injection.
d) Mean±SE.
e) Significantly different from control group ($P<0.001$).
f) Significantly different from control group ($P<0.05$).

Table III. Number and Incidence of Colon Tumors in Each Experimental Group

Diet	No. of tumors		Carcinomas		Total tumors[a]	
	Carcinoma	Adenoma	Carcinomas/rat	% of rat with carcinomas	Tumors/rat	% of rat with tumors
Control (n=18)	25	4	1.39±0.24[b]	83.3	1.61±0.27	83.3
Citrus pectin (n=20)	19	2	0.95±0.28	55.0	1.05±0.29	55.0
Apple pectin (n=24)	9	2	0.38±0.17[c]	29.2[d]	0.46±0.18[d]	37.5[c]

a) Carcinoma and adenoma.
b) Mean±SE.
c) Significantly different from control group ($P<0.01$).
d) Significantly different from control group ($P<0.001$).

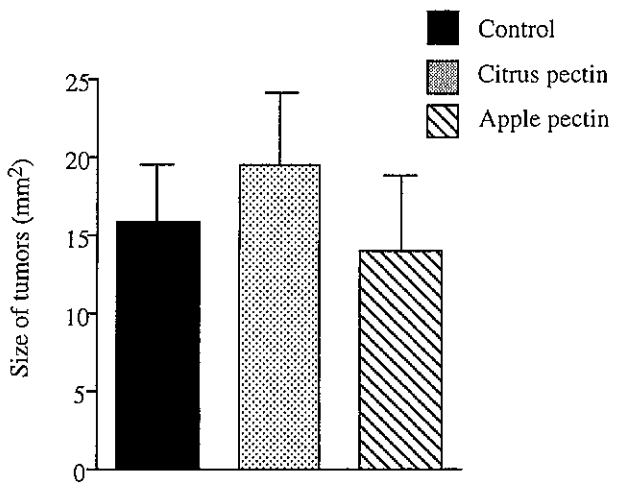

Fig. 1. The mean size of tumors in each group was estimated by using the following equation: Tumor size=$a \times b$, where a and b are the length and width (mm), respectively. All values are the mean±SE.

Table IV. Effect of Pectin on Fecal Moisture

Weeks[a]	Fecal moisture (%)		
	Control	Citrus pectin	Apple pectin
0[b]	58.7±1.9[c]	70.4±2.6[d]	70.9±3.9[e]
11	62.2±3.2	63.7±1.9	68.3±1.1
19	65.8±0.9	61.7±1.6	66.7±1.7
30	63.8±2.5	67.8±3.9	74.4±4.7

a) Weeks after the first AOM injection.
b) Two weeks after starting diet administration.
c) Mean±SE.
d) Significantly different from control group ($P<0.01$)
e) Significantly different from control group ($P<0.05$)

total tumors and carcinomas per rat in the AP group (0.46±0.18, 0.38±0.17) were statistically significantly different from those in the control group (1.61±0.27, 1.39±0.24) ($P<0.001$, $P<0.01$).

The mean size of tumors in each group is shown in Fig. 1. There was no difference in tumor size among these three groups.

Histology The majority of the colonic tumors were well-differentiated tubular adenocarcinomas, and in a few cases, signet-ring cell carcinomas were found invading the lamina propria or the serosa. There were no histological abnormalities in the liver, kidney, or spleen or rats fed the pectin diet.

Fecal moisture At week 0, the fecal moisture in the citrus and apple pectin groups was significantly increased compared with that in the control group ($P<0.01$, $P<0.05$, respectively), but there was no difference from the 11th week on (Table IV).

Fecal β-glucuronidase activity Fig. 2 shows β-glucuronidase activities in fresh fecal specimens on the 0, 11th, 19th and 30th weeks after the first AOM injection. At week 0 (at 2 weeks after starting the diet), a significant decrease of activity was seen in the apple pectin group compared with that in the control group: the activity in the apple pectin group was 1/10 of that in the control group. However, from the 11th week on, the activity was higher in both pectin diet groups compared with that in the control group ($P<0.05$ or less).

Fecal β-glucosidase activity From week 0, β-glucosidase activity tended to be lower in the citrus pectin group ($P<0.1$) compared with that in the control group, while the apple pectin group had a significantly lower β-glucosidase activity ($P<0.05$ or less) than did the control group (Fig. 3).

Fecal tryptophanase activity In the citrus pectin group, tryptophanase activity was decreased compared with that in the control group at weeks 0 and 30, but not in the intervening period. In the apple pectin group, the activity tended to be decreased compared with that in the control group (Fig. 4).

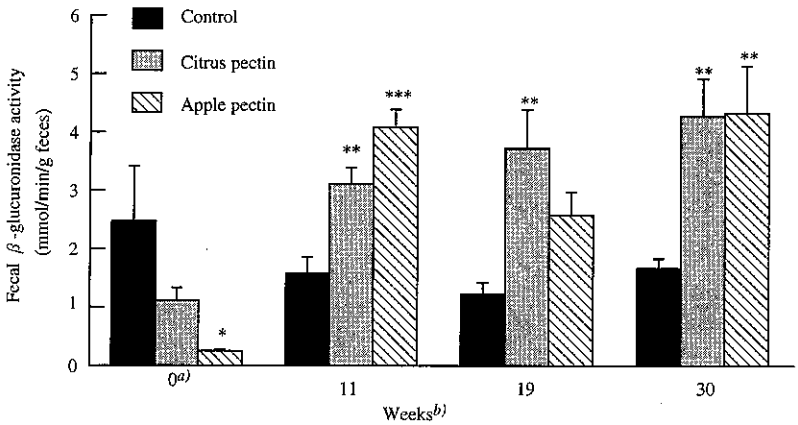

Fig. 2. Effect of pectin on fecal β-glucuronidase activity in AOM-treated rats. β-Glucuronidase assay was performed as described in "Materials and Methods." *a*) Two weeks after starting diet administration. *b*) Weeks after the first AOM injection. All values are the mean±SE; n=5 for each group. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs. control.

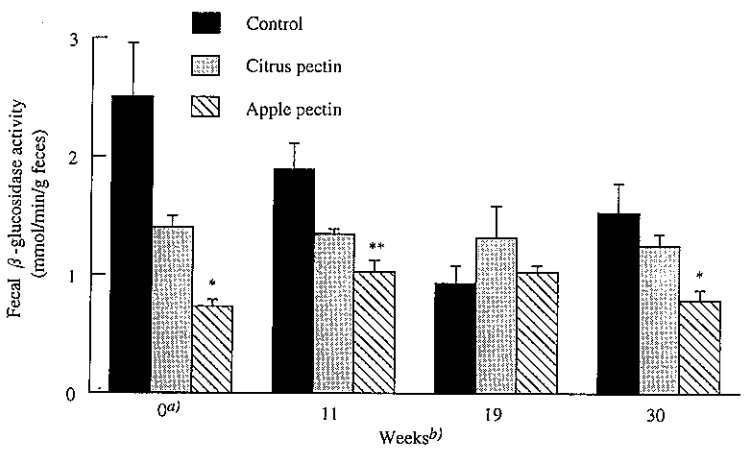

Fig. 3. Effect of pectin on fecal β-glucosidase activity in AOM-treated rats. β-Glucosidase assay was performed as described in "Materials and Methods." *a*) Two weeks after starting diet administration. *b*) Weeks after the first AOM injection. All values are the mean±SE; n=5 for each group. * $P<0.05$, ** $P<0.01$ vs. control.

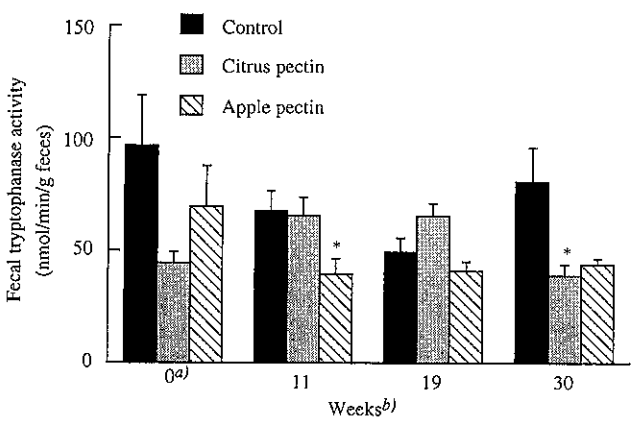

Fig. 4. Effect of pectin on fecal tryptophanase activity in AOM-treated rats. Tryptophanase activity was determined by the method of Demoss and Moser.[18] *a*) Two weeks after starting diet administration. *b*) Weeks after the first AOM injection. All values are the mean±SE; n=5 for each group. * $P<0.05$ vs. control.

DISCUSSION

Pectin is a partially methoxylated polymer of galacturonic acid obtained from fruit. It has been used as a gelling agent in the preparation of jam and is a water-soluble dietary fiber. Jacobs and Lupton reported that water-soluble fibers enhance rat colon carcinogenesis,[4] and several reports have appeared on the action of pectin (mostly citrus pectin) in experimental colon carcinogenesis.[2-9] We reported that apple pectin inhibited AOM-induced colon carcinogenesis in rats.[10,11] Apple pectin has stronger bacteriostatical action against pathogenic bacteria compared with citrus pectin,[12] and may markedly alter the composition of the intestinal bacterial flora. The intestinal flora is a metabolically active group of organisms that produce enzymes catalyzing the metabolism of procarcinogens and formation of tumor promoters in the colon, and it may play a significant role in the pathogenesis of colon cancer.[13-16] The bacterial en-

zymes, including β-glucosidase, β-glucuronidase and tryptophanase, are potentially important in the generation of toxicants and carcinogens.[19,20] Therefore, the present study was undertaken to determine the modifying effect of different types of pectin, apple and citrus pectin, on fecal bacterial enzymes.

The results of the present study indicate that the induction of colon neoplasms by AOM was more potently inhibited by apple pectin than by citrus pectin. We are not aware of any previous study on the potential colon tumor-inhibitory effect of apple pectin.

In the present study, the rats fed a diet containing pectin showed about 15% lower body weight compared with those fed a control diet. The question arises as to whether the inhibitory effect of apple pectin is related to the lower body weight gain of rats as a result of pectin feeding. If this were so, the rats fed the citrus pectin should have the same tumor incidence and number of colon tumors as the rats fed the apple pectin, because these two groups did not differ in body weight gain. In this study, this was not the case. Therefore, the depressed weight gain cannot by itself explain the modifying effect of apple pectin on AOM-induced colon carcinogenesis.

At 2 weeks after starting diet administration, the fecal moisture in both pectin groups was significantly increased. The feces-softening effect of pectin feeding may be significant.

After p.o. intake, the plant product cycasin reaches the large intestine where bacterial β-glucosidase hydrolyzes it to the toxic metabolite, methylazoxymethanol (MAM).[21–24] In the present study, from week 0, β-glucosidase activity tended to be lower in the citrus pectin group than in the control group. The apple pectin group had a significantly lower β-glucosidase activity than did the control diet group (Fig. 3). Reduced β-glucosidase activity following cellulose administration was also reported.[6,25] Thus, luminal carcinogens derived from dietary β-glycosides might be reduced by dietary fibers.

Chung *et al.*[26] suggested that the findings of higher tryptophanase activity and tryptophan concentration in the feces of animals fed an all-meat diet lend some support to the epidemiologic association of colon cancer with high meat intake. In our study, fecal tryptophanase activity tended to be decreased in the apple pectin group compared with that in the control group (Fig. 4). Reduced tryptophan metabolites in the colon might be related to the inhibitory effect of apple pectin on colon carcinogenesis.

Bauer *et al.*[7] found an increased incidence of dimethylhydrazine-induced colorectal tumors and fecal β-glucuronidase activity in male Sprague-Dawley rats fed a diet containing 6.5% pectin. In a later study,[3] however, they reported that bacterial β-glucuronidase activity in feces and the number of tumors were the same in 5% pectin-fed rats and controls. Freeman,[6] on the other hand, reported that the number of dimethylhydrazine-induced colon neoplasia in pectin-fed rats was unchanged, although pectin-fed rats had increased fecal β-glucuronidase activity. It seems likely that higher fecal β-glucuronidase activity in the pectin-fed animals would be associated with a higher tumor incidence. In the present study, at week 0 (2 weeks after starting the diet), the β-glucuronidase activity in the apple pectin group, but not the citrus pectin group, was 1/10 of that of the control group (Fig. 2). After the 11th week, this enzyme activity increased in pectin-fed rats, as previously reported.[6] We presume that the microflora may be altered by continuous pectin feeding and AOM injection, which caused the elevation of the β-glucuronidase activity. In this experiment, we did not investigate the microflora, so examination of the microflora at each stage remains necessary. Bacterial β-glucuronidase is believed to be largely responsible for the hydrolysis of glucuronide conjugates in the gut and thus to be important in the generation of toxic and carcinogenic substances.[14,19,20,27] AOM, the carcinogen used in the present study, is hydroxylated to MAM in the liver, and Weisburger postulated[28,29] that MAM is conjugated with glucuronic acid in the liver and excreted into the bile. The bacterial flora of the intestine may then convert this compound back to the aglycone MAM by the action of β-glucuronidase. Thus, the major decrease of β-glucuronidase activity in the apple pectin group at week 0 may decrease the production of MAM in the colon, which should inhibit tumor initiation. At week 0, the β-glucuronidase activity in the apple pectin group was decreased more than that in the citrus pectin group, and indeed, the inhibition of colon tumor multiplicity was more pronounced with apple pectin than with citrus pectin.

In a recent study, we examined β-glucuronidase activity during the initiation phase every week and found that the lower β-glucuronidase activity in the apple pectin group gradually increased to reach that in the control group at 6 weeks after starting the diet (data not shown). Takada *et al.*[30] induced colon carcinomas with AOM and studied the effect of a β-glucuronidase inhibitor on the colon carcinogenesis. They reported that fewer tumors were found in the rats given a β-glucuronidase inhibitor at the same time as AOM, but the rats given a β-glucuronidase inhibitor after AOM treatment had slightly more tumors than the control. Thus, it seems likely that the decrease of β-glucuronidase activity in the apple pectin group during the AOM injection is related to the inhibitory effect of apple pectin on AOM-induced colon carcinogenesis.

In summary, we have demonstrated that apple pectin is a potent inhibitor of AOM-induced colon carcinogenesis in rats, and we suggest that the decrease of fecal β-

glucuronidase level during the initiation stage of carcinogenesis plays an important role in this.

ACKNOWLEDGMENTS

We wish to thank Dr. Takeshi Sakai, 2nd Department of Pathology, Toyama Medical and Pharmaceutical University, for his comments on the histological classification of the rat neoplastic tissues, Miss Sanae Hirota for her technical assistance and Mrs. Miki Shoji for her careful reading of the manuscript and helpful suggestions. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, and a 1993 grant from the Tamura Foundation for Promotion of Science and Technology in Japan.

(Received January 9, 1995/Accepted March 17, 1995)

REFERENCES

1) Burkitt, D. P. Epidemiology of cancer of the colon and rectum. *Cancer,* **28,** 3–13 (1971).
2) Watanabe, K., Reddy, B. S., Weisburger, J. H. and Kritchevsky, D. Effect of dietary alfalfa, pectin, and wheat bran on azoxymethane or methylnitrosourea-induced colon carcinogenesis in F344 rats. *J. Natl. Cancer Inst.,* **63,** 141–145 (1979).
3) Bauer, H. G., Asp, N. G., Dahlqvist, A., Fredlund, P. E., Nyman, M. and Oste, R. Effects of two kinds of pectin and guar gum on 1,2-dimethylhydrazine initiation of colon tumors and fecal β-glucuronidase activity in the rat. *Cancer Res.,* **41,** 2518–2523 (1981).
4) Jacobs, L. R. and Lupton, J. R. Relationship between colonic luminal pH, cell proliferation, and colon carcinogenesis in 1,2-dimethylhydrazine treated rats fed high fiber diets. *Cancer Res.,* **46,** 1727–1734 (1986).
5) Masaki, K. Effect of dietary fibers on colon carcinogenesis induced by 1,2-dimethylhydrazine in rat. *J. Wakayama Med. Soc.,* **44,** 351–364 (1993) (in Japanese).
6) Freeman, H. J. Effects of differing purified cellulose, pectin, and hemicellulose fiber diets on fecal enzymes in 1,2-dimethylhydrazine-induced rat colon carcinogenesis. *Cancer Res.,* **46,** 5529–5532 (1986).
7) Bauer, H. G., Asp, N. G., Oste, R., Dahlqvist, A. and Fredlund, P. E. Effect of dietary fiber on the introduction of colorectal tumors and fecal β-glucuronidase activity in the rat. *Cancer Res.,* **39,** 3752–3756 (1979).
8) Freeman, H. J., Spiller, G. A. and Kim, Y. S. A double-blind study on the effects of differing purified cellulose and pectin fiber diets on 1,2-dimethylhydrazine-induced rat colonic neoplasia. *Cancer Res.,* **40,** 2661–2665 (1980).
9) Heitman, D. W., Hardman, W. E. and Cameron, I. L. Dietary supplementation with pectin and guar gum on 1,2-dimethylhydrazine-induced colon carcinogenesis in rats. *Carcinogenesis,* **13,** 815–818 (1992).
10) Tazawa, K., Ookami, H., Yamashita, I., Shimizu, T., Fujimaki, M., Murai, K., Kobashi, K. and Honda, T. Effect of apple pectin on azoxymethane-induced colon carcinogenesis — fecal enzyme activities and prostaglandin E_2 level in colonic mucosa. *In* "Recent Advantage in Management of Digestive Cancers," ed. T. Takahashi, pp. 471–473 (1993). Springer-Verlag, Tokyo.
11) Ookami, H., Tazawa, K., Okamoto, M., Yamashita, I., Masuyama, K., Shimizu, T., Yamamoto, K., Katsuyama, S., Takemori, S., Arai, H., Sakamoto, T., Yamashita, Y., Maeda, M., Honda, T. and Fujimaki, M. Effect of apple pectin on azoxymethane-induced colon carcinogenesis (first report); prostaglandin E2 level in colonic mucosa and portal vein. *J. Jpn. Res. Soc. Gastroenterol. Carcinog.,* **5,** 91–94 (1993) (in Japanese).
12) Tazawa, K. Bacteriostatical properties of skin barriers. *Proc. 7th Bienn. Congr. World Council of Enterostomal Therapists,* 37–41 (1988).
13) Simon, G. L. and Gorbach, S. L. Intestinal flora in health and disease. *Gastroenterology,* **86,** 174–193 (1984).
14) Goldin, B. R. and Gorbach, S. L. The relationship between diet and rat fecal bacterial enzymes implicated in colon cancer. *J. Natl. Cancer Inst.,* **57,** 371–375 (1978).
15) Prizont, R. and Konisberg, N. Identification of bacterial glycosidases in rat fecal contents. *Dig. Dis. Sci.,* **26,** 773–777 (1981).
16) Scheline, R. R. Metabolism of foreign compounds by gastrointestinal microorganisms. *Pharmacol. Rev.,* **25,** 451–523 (1973).
17) Akao, T., Akao, T. and Kobashi, K. Glycyrrhizin β-D-glucuronidase of *Eubacterium* sp. from human intestinal flora. *Chem. Pharm. Bull.,* **35,** 705–710 (1987).
18) Demoss, R. D. and Moser, K. Tryptophanase in diverse bacterial species. *J. Bacteriol.,* **98,** 167–171 (1969).
19) Williams, R. T. Toxicologic implications of biotransformation by intestinal microflora. *Toxicol. Appl. Pharmacol.,* **23,** 769–781 (1972).
20) Chipman, J. K. Biles as a source of potential reactive metabolites. *Toxicology,* **25,** 99–111 (1982).
21) Laqueur, G. L. Carcinogenic effects of cycad meal and cycasin, methylazoxymethanol glycoside, in rats and effects of cycasin in germfree rats. *Fed. Proc.,* **23,** 1386–1388 (1964).
22) Laqueur, G. L. Contribution of intestinal macroflora and microflora to carcinogenesis. *In* "Carcinoma of the Colon and Antecedent Epithelium," ed. W. J. Burdette, pp. 305–313 (1970). Charles C. Thomas Publisher, Springfield, IL.
23) Laqueur, G. L. and Spatz, M. Oncogenicity of cycasin and methylazoxymethanol. *Gann Monogr. Cancer Res.,* **17,** 189–204 (1975).
24) Narisawa, T. and Nakano, H. Carcinoma of the large intestine of rats induced by rectal infusion of methylazoxymethanol. *Gann,* **64,** 93–95 (1973).

25) Prizont, R. Influence of high dietary cellulose on fecal glucosidases in experimental rat colon carcinogenesis. *Cancer Res.*, **44,** 557–561 (1984).
26) Chung, K. T., Fulk, G. E. and Slein, M. W. Tryptophanase of fecal flora as a possible factor in the etiology of colon cancer. *J. Natl. Cancer Inst.*, **54,** 1073–1078 (1975).
27) Weisburger, J. H., Grantham, P. H., Horton, R. E. and Weisburger, E. K. Metabolism of the carcinogen N-hydroxy-N-2-fluorenylacetamide in germfree rats. *Biochem. Pharmacol.*, **19,** 151–162 (1970).
28) Weisburger, J. H. Colon carcinogens: their metabolism and mode of action. *Cancer,* **28,** 60–70 (1971).
29) Weisburger, J. H. Chemical carcinogens and their mode of action in colonic neoplasma. *Dis. Colon Rectum,* **16,** 431–437 (1973).
30) Takada, H., Hirooka, T., Hiramatsu, Y. and Yamamoto, M. Effect of β-glucuronidase inhibitor on azoxymethane-induced colonic carcinogenesis in rats. *Cancer Res.*, **42,** 331–334 (1982).