

Effect of Dietary Undegraded Carrageenan on Colon Carcinogenesis in F344 Rats Treated with Azoxymethane or Methylnitrosourea¹

Kenshi Watanabe, Bandaru S. Reddy,² Ching Q. Wong, and John H. Weisburger

Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, New York 10595

ABSTRACT

The effect of dietary undegraded carrageenan (Viscarin 402) on colon carcinogenesis was studied in female inbred F344 rats. Weanling rats were fed semipurified diets containing 0 or 15% undegraded carrageenan. At 7 weeks of age, all animals except controls were given azoxymethane (AOM) s.c. at a dose rate of 8 mg/kg body weight per week for 10 weeks or methylnitrosourea (MNU) intra-rectally at a dose level of 2 mg/rat twice a week for 3 weeks. The AOM groups were autopsied 40 weeks and the MNU groups 30 weeks after the first injection. No tumors were induced in the colon or in other organs of untreated rats fed the control diet. One untreated rat fed the carrageenan diet showed a colon adenoma. The animals fed the carrageenan diet and treated with AOM or MNU had a higher incidence of colorectal tumors (number of rats with colorectal tumors and number of tumors per tumor-bearing rat) than did those fed the control diet and treated similarly. The undegraded carrageenan (Viscarin 402) in the diet had an enhancing effect in colorectal carcinogenesis in rats evoked by AOM or MNU.

INTRODUCTION

Animal models have been used to study multiple environmental factors involved in the pathogenesis of large bowel cancer as well as to elucidate the mechanism of colon carcinogenesis by a variety of chemicals (3, 5, 12, 13).

Carrageenans are complex polysaccharides prepared from the red marine algae and are widely used in the food industry (14). A series of studies in England with degraded carrageenan, C-16, showed that this product could induce ulceration of the cecum and proximal colon in laboratory animals (8, 9, 19). In the United States undegraded carrageenan is being used particularly in dairy products as a stabilizer, gelling agent, and viscosity control agent. Carrageenan can absorb much larger quantities of water than can commonly used fibers such as wheat bran, pectin, or alfalfa and acts as a bulking agent.

Because of recent interest in dietary fiber and fecal bulk as protection against colon cancer, we have studied the modifying effect of undegraded carrageenan on AOM³- and MNU-induced colon carcinogenesis in rats fed a high-fat diet.

MATERIALS AND METHODS

Weanling inbred female F344 rats were obtained from Charles River Breeding Laboratory (Wilmington, Mass.). At 5 weeks of age, animals were randomly divided into 6 groups and fed *ad libitum* one of the semipurified diets containing 0 and 15% undegraded carrageenan (Table 1). Native carrageenan (Viscarin 402) was purchased from Marine Colloids, Inc. (Springfield, N. J.), and the special diets were prepared according to our specifications in pelleted form by Bio-Serv, Inc. (Frenchtown, N. J.). All animals were housed in plastic cages and maintained in a temperature- and humidity-controlled room. At 7 weeks of age, 30 rats in each dietary group, except the controls, received 1 weekly s.c. injection of AOM for 10 weeks (8 mg per kg body weight per week). In another series, 30 rats on each diet, except the controls, were given i.r. instillations of MNU (2 mg/rat twice a week for 3 weeks). MNU and AOM were freshly dissolved in 0.1 M phosphate buffer (pH 6.5) and 0.9% NaCl solution, respectively. Controls from each dietary group were given an equal volume of phosphate buffer or 0.9% NaCl solution. These levels of carcinogens and period of administration were chosen based on our past experience, so that the modifying effect of carrageenan, if any, could be readily observed.

Animals that were dying or becoming moribund were necropsied. All animals in the MNU group were autopsied 30 weeks after first injection, and in the AOM group as well as 15 untreated rats from each dietary group, they were autopsied after 40 weeks. All organs, including the intestines, were examined grossly and histologically for the number and types of tumors. Tissues were fixed in 10% buffered formalin; the sections were stained with hematoxylin and eosin.

RESULTS

Chart 1 shows the body weight gain of rats fed the 2 diets. Animals fed carrageenan diet gained less weight than did the animals on the control diet. Feed intakes measured in each dietary group during the third week on the experiment showed that all animals consumed approximately equal amounts (8.6 and 9.9 g/rat/day on control and carrageenan diets, respectively).

Table 2 summarizes the AOM-induced tumor incidence in rats fed control and carrageenan diets. No tumors were found in the colon or in other organs of untreated rats fed the control diet; however, one untreated rat consuming a carrageenan diet showed a colon adenoma.

The number of rats with AOM-induced colon tumors were significantly higher with the carrageenan diet compared with the control diet. The incidence of colon tumors (total

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² To whom requests for reprints should be addressed.

³ The abbreviations used are: AOM, azoxymethane; MNU, methylnitrosourea; i.r., intrarectal.

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Table 1
Percentage composition of diets

Ingredients	Control diet	Carrageenan diet
Soybean protein	22.0	22.0
DL-Methionine (feed grade)	0.3	0.3
Corn starch	34.2	24.2
Dextrose	12.0	7.0
Alphacel	5.0	5.0
Undegraded carrageenan (Viscarin 402)	0	15.0
Corn oil	20.0	20.0
Salt mixture ^a	4.5	4.5
Vitamin diet fortification mixture ^b	2.0	2.0

^a The salt mixture was formulated according to Rogers and Harper (15) and contained the following composition (g/kg salt mixture): ammonium molybdate·4H₂O 0.03; calcium carbonate, 292.90; calcium phosphate·2H₂O, 430; cupric sulfate, 1.56; ferric citrate·6H₂O, 6.23; magnesium sulfate·7H₂O, 99.80; manganese sulfate·H₂O, 1.21; potassium iodide, 0.005; potassium phosphate, 343.10; sodium chloride, 250.60; sodium selenite·5H₂O, 0.02; zinc chloride, 0.20.

^b Vitamin diet fortification mixture was obtained from ICN Pharmaceuticals, Inc., Cleveland, Ohio and contained the following composition (g/kg vitamin mixture): vitamin A concentrate (200,000 IU/g), 9.0; vitamin D concentrate (400,000 IU/g), 0.50; α-tocopherol, 10.0; inositol, 10.0; choline chloride, 150.0; menadione, 4.50; niacin, 9.0; riboflavin, 2.0; pyridoxine hydrochloride, 2.0; thiamine hydrochloride, 2.0; calcium pantothenate, 6.0; biotin, 0.04; folic acid, 0.18; vitamin B₁₂, 0.0027. The ingredients are triturated and made to 1 g with dextrose.

tumors per tumor-bearing rat) was higher in animals fed carrageenan diet compared with incidence of colon tumors in those fed a control diet (Table 2). All rats on the carrageenan diet had more multiple colon tumors than did those on a control diet (Fig. 1).

Table 3 summarizes the colon tumor incidence in rats treated with MNU. The incidence and multiplicity of MNU-induced colon tumors in rats fed the carrageenan diet was higher than those in rats fed control diet (Table 3; Fig. 2).

In rats given the carrageenan diet, the AOM-induced colon tumors were mainly localized in the distal part within 4 cm from the anus, whereas in rats fed control diet, they were distributed mainly 2 to 10 cm from the anus (Chart 2). On the other hand the MNU-induced colon tumors in the carrageenan-fed rats were localized in the distal part of the colon within 4 cm from the anus, and in the rats fed a control diet, they were distributed within 7 cm from the anus. The colon adenoma in the untreated rat was localized 6 cm from the anus.

Grossly, the colon tumors were polypoid and sessile. The size of colon tumors in rats was also larger with the carrageenan than with the control diet. In rats fed the control diet, 84% of colon tumors were less than 4 mm in diameter, whereas in rats fed carrageenan diet, 52% of colon tumors were larger than 4 mm.

The standards for histological diagnosis of intestinal tumors were described by Muto et al. (10). Histologically,

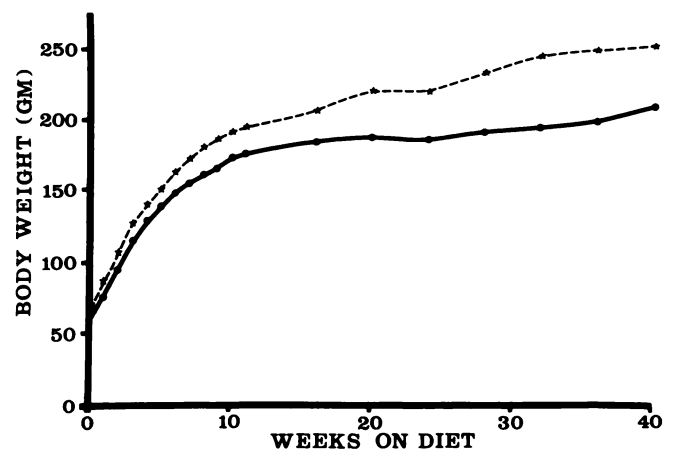


Chart 1. Body weights of female F344 rats fed diets containing 0 or 15% undegraded carrageenan (Viscarin 402). The body weights of 4-week-old rats were recorded for 40 weeks. Control diet (x); carrageenan diet (●).

Table 2
Intestinal tumor incidence in female F344 rats fed a control or undegraded carrageenan diet and treated with AOM

Diets	Carcinogen	No. of rats	Colon tumors		Tumors/rat	Tumor classification (total tumors)			
			Incidence			Colon			Duodenum (adenocarcinoma)
			No.	%		Adenoma	Focal carcinoma	Adenocarcinoma	
Control diet	AOM	30	17 ^a	57 ^a	1.5	14	5	6	2
Carrageenan diet	AOM	26	26	100	11.3	158	76	59	0
Control diet		15	0	0	0	0	0	0	0
Carrageenan diet		15	1	7	1	1	0	0	0

^a Significantly different from the carrageenan diet by χ^2 test ($p < 0.01$, or better).

Table 3
Colon tumor incidence in female F344 rats fed a control or undegraded carrageenan diet and treated with MNU

Diets	No. of rats	Incidence		Tumors/rat	Tumor classification (total tumors)		
		No.	%		Adenoma	Focal carcinoma	Adenocarcinoma
		Control diet	29		20 ^a	69 ^a	1.5
Carrageenan diet	29	29	100	4.4	88	21	19

^a Significantly different from the carrageenan diet by χ^2 test ($p < 0.01$, or better).

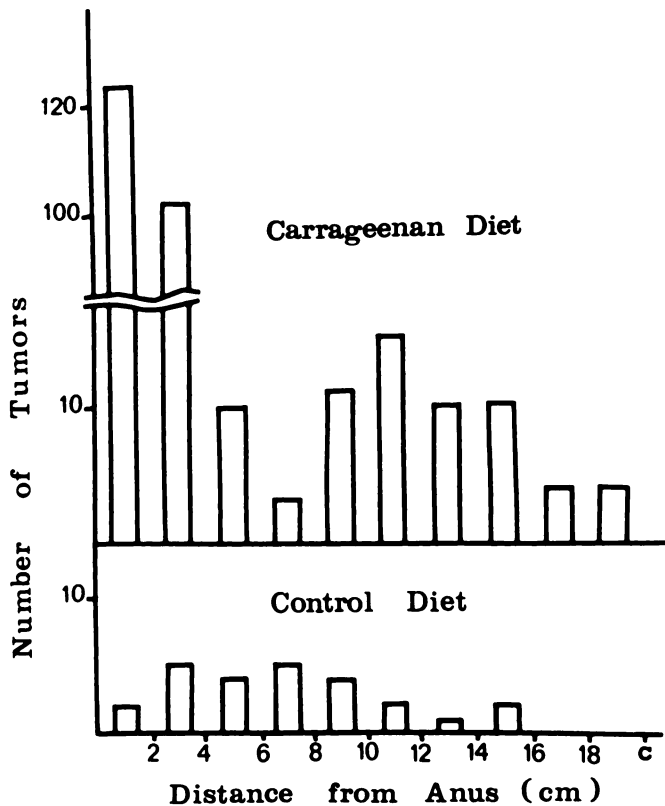


Chart 2. Number and distribution as a function of distance from anus of AOM-induced colorectal tumors in female F344 rats fed diets containing 0 or 15% undegraded carrageenan. At 7 weeks of age, animals in each dietary group were given 1 weekly s.c. injection of AOM for 10 weeks at a dose level of 8 mg per kg body weight per week and autopsied 40 weeks later.

the adenomas classified in the study were benign tumors of intestinal glandular structure lining with a slight or moderate atypical epithelium. Focal carcinomas showed considerable irregularity of the tabules or villi with severe atypical epithelium. Adenocarcinomas were frank malignant tumors showing invasion across the line of the muscularis mucosa and usually well differentiated. Four of the colon tumors were signet ring cell carcinomas infiltrating into the submucosal layer. One well-differentiated adenocarcinoma showed the metastasis to the regional lymph node, and one animal showed the peritonitis carcinomatosa.

The duodenal tumors induced by AOM were sessile and histologically well-differentiated adenocarcinomas deeply invading into the intestinal wall. One animal had peritonitis carcinomatosa with dissemination of tumor cells into the abdominal cavity.

All untreated rats fed the carrageenan diet showed chronic inflammatory changes in the large intestine composed of edematous thickening and aggregation of macrophages in the lamina propria and submucosa. In few animals mucosal surface of the distal part of the colon showed a grayish-white, coating-like appearance. No malignant tumors were found with the exception of one animal showing an adenomatous polyp in the colon.

DISCUSSION

Although methionine was probably limiting in our experimental diets that contained 0.3% supplemental methionine,

the body weights of animals fed the control diet were normal. However, the animals fed a 15% carrageenan diet showed a 10 to 20% lower body weight compared with those fed a control diet. In an earlier study no growth retardation was noted in rats fed 10% undegraded carrageenan in the diet, but when the carrageenan was added to the diet at 25% level, the animals gained less weight compared with those fed a cellulose diet (7). Perhaps the depression of nitrogen absorption observed in studies by Hawkins and Yaphe (7) as well as decreased caloric intake explain the slower growth rate.

Degraded carrageenan given in drinking water or by stomach tube for periods ranging from 30 days to 1 year caused pin-head-sized ulcers of the cecum and colon in rats, mice, guinea pigs, and rabbits (4, 6, 8, 9, 19). Although some species of animals fed the undegraded carrageenan appeared to be healthy with no adverse effect (2, 8, 11), guinea pigs had ulceration in the large intestine (1, 6).

Our results indicate that a diet containing undegraded carrageenan produced histopathological changes in the large intestine of rats. One rat had an adenomatous polyp in the colon. Although the possibility exists that the single adenoma in an untreated rat fed carrageenan diet may be spontaneous, such lesions are rarely seen in untreated rats fed the control diet. Undegraded carrageenan (Viscarin 402), used in this study, did not show any mutagenic activity in the Ames *Salmonella* system (unpublished results), but further studies are required to test whether carrageenan itself is tumorigenic in animals.

The rats fed a diet containing undegraded carrageenan were more susceptible to colon tumor induction by AOM or MNU than were rats fed a diet without carrageenan. Equivalent or higher levels of cellulose, as alphacel, had virtually no effect in colon carcinogenesis (17). Our recent study indicated that the diets containing 15% wheat bran or pectin had no effect on MNU-induced colon carcinogenesis, whereas these diets greatly inhibited colon tumor incidence induced by AOM compared to a diet containing 15% alfalfa or 5% cellulose (18). Wilson *et al.* (20) showed that the rats fed a diet containing 15% wheat bran developed fewer 1,2-dimethylhydrazine-induced colon tumors than did those fed a control diet containing 5% cellulose. In this study irrespective of the type of colon carcinogen, namely AOM and MNU which differ in mechanism of activation, the animals fed a carrageenan diet always had a higher incidence of colorectal tumors than did controls. The question arises whether the observed results are related to the dietary carrageenan *per se* or to lower body weight gain as a result of carrageenan feeding. Since it is known that calorie restriction and consequent weight loss could decrease the tumor incidence (16), it could be interpreted that these results are specifically not related to the feeding of carrageenan. If this were so the rats fed the carrageenan diet should develop fewer colon tumors than those fed control diet. In this study this was not the case. In addition, our recent study indicated that although the rats fed a diet containing 15% pectin gained about 25% less weight than did the rats on a control diet, the incidence of AOM-induced colon tumors was lower in rats fed pectin-containing diet than in rats fed a control diet (18). Thus, the observed results are most probably related to carrageenan feeding.

REFERENCES

1. Abraham, R., Fabian, R. J., Golberg, L., and Coulston, F. Role of Lysosomes in Carrageenan-Induced Cecal Ulceration. *Gastroenterology*, 67: 1169-1181, 1974.
2. Benitz, K. F., Golberg, L., and Coulston, F. Intestinal Effects of Carrageenans in the Rhesus Monkey. *Food Cosmet. Toxicol.*, 11: 565-575, 1973.
3. Bralow, S. P., and Weisburger, J. H. Experimental Carcinogenesis in the Digestive Organs. *Clin. Gastroenterology* 5: 527-542, 1976.
4. Fabian, R. J., Abraham, R., Coulston, F., and Golberg, L. Carrageenan-Induced Squamous Metaplasia of the Rectal Mucosa in the Rat. *Gastroenterology* 65: 265-276, 1973.
5. Fiala, E. S. Investigations into the Metabolism and Mode of Action of the Colon Carcinogens 1,2-Dimethylhydrazine and Azoxymethane. *Cancer*, 40: 2436-2445, 1977.
6. Grasso, P., Sharratt, M., Carpanini, F. M. B., and Gangolli, S. D. Studies on Carrageenan and Large-Bowel Ulceration in Mammals. *Food Cosmet. Toxicol.*, 11: 555-564, 1973.
7. Hawkins, W. W., and Yaphe, W. Carrageenan as a Dietary Constituent for the Rat: Faecal Excretion, Nitrogen Absorption, and Growth. *Can. J. Biochem.*, 43: 479-484, 1965.
8. Marcus, R., and Watt, J. Seaweeds and Ulcerative Colitides in Laboratory Animals. *Lancet*, 2: 489-490, 1969.
9. Marcus, R., and Watt, J. Colonic Ulceration in Young Rats Fed Degraded Carrageenan. *Lancet*, 2: 765-766, 1971.
10. Muto, T., Bussey, H. J. R., and Morson, B. C. The Evolution of Cancer of the Colon and Rectum. *Cancer*, 36: 2251-2270, 1975.
11. Poulsen, E. Short-Term Peroral Toxicity of Undegraded Carrageenan in Pigs. *Food Cosmet. Toxicol.*, 11: 219-277, 1973.
12. Reddy, B. S. Carcinogenesis of the Colon and Rectum. In: W. E. Enker (ed.), *Carcinoma of the Colon and Rectum*, pp. 326-343. Chicago: Year Book Medical Publishers, Inc., 1978.
13. Reddy, B. S., Watanabe, K., and Weisburger, J. H. Effect of High-Fat Diet on Colon Carcinogenesis in F344 Rats Treated with 1,2-Dimethylhydrazine, Methylazoxymethanol Acetate, or Methylnitrosourea. *Cancer Res.*, 37: 4156-4159, 1977.
14. Rees, W. M. Phycocolloids and Marine Colloids. In: F. E. Firth (ed.), *Encyclopedia of Marine Resources*, pp. 521-527. New York: Van Nostrand-Rheinhold Co., 1969.
15. Rogers, Q. R., and Harper, A. E. Amino Acid Diets and Maximal Growth in the Rat. *J. Nutr.*, 87: 267-273, 1965.
16. Tannenbaum, A., and Silverstone, H. Nutrition and Genesis of Tumors. In: R. W. Raven (ed.), *Cancer*, Vol. 1, pp. 306-334. London: Butterworth & Co., Ltd., 1957.
17. Ward, J. M., Yamamoto, R. S., and Weisburger, J. H. Cellulose Dietary Bulk and Azoxymethane-Induced Intestinal Cancer. *J. Natl. Cancer Inst.*, 51: 713-715, 1973.
18. Watanabe, K., Reddy, B. S., and Kritchevsky, D. Effect of Various Dietary Fibers and Food Additives on Azoxymethane or Methylnitrosourea-Induced Colon Carcinogenesis in Rats. *Federation Proc.*, 37: 262, 1978.
19. Watt, J., and Marcus, R. Carrageenan-Induced Ulceration of the Large Intestine in the Guinea Pig. *Gut*, 12: 164-171, 1971.
20. Wilson, R. B., Hutcheson, D. P., and Wideman, L. Dimethylhydrazine-Induced Colon Tumors in Rats Fed Diets Containing Beef Fat or Corn Oil with and without Wheat Bran. *Am. J. Clin. Nutr.*, 30: 176-181, 1977.

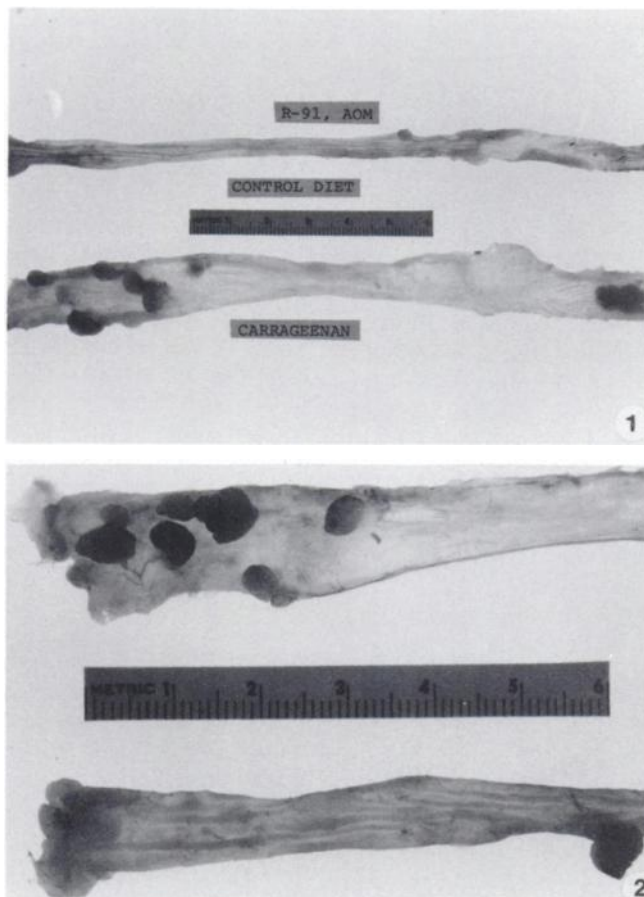


Fig. 1. AOM-induced colorectal tumors in female F344 rats fed a control diet (upper) or a carrageenan diet (lower) containing 0 or 15% undegraded carrageenan, respectively.

Fig. 2. MNU-induced colorectal tumors in female F344 rats fed a control diet (lower) or a carrageenan diet (upper) containing 0 or 15% undegraded carrageenan, respectively.