UNIVERSITY OF ILLINOIS
AT CHICAGO

Section of General Internal Medicine (MC 718)
Department of Medicine
840 South Wood Street, Room 440 CSN
Chicago, Illinois 60612-7315

April 27, 2012

National Organic Standards Board
c/o USDA – AMS – NOP
Washington, D.C. 20250-0268

Re: Carrageenan Sunset Review

Dear Members of the National Organic Standards Board:

I am a physician-scientist at the University of Illinois College of Medicine who has been studying the effects of carrageenan in human cells and in animal models for almost two decades. With collaborators, I have published 18 peer-reviewed papers that address the biological effects of carrageenan. Most of this work has been funded by the National Institutes of Health and the Veterans' Administration.

In this comment, three major points are addressed. These are: 1) exposure to carrageenan causes inflammation which is harmful; 2) the amount of carrageenan consumed in the human diet is sufficient to cause inflammation; 3) both undegraded and degraded carrageenan cause inflammation.

Carrageenan is used in food due to its potent chemical effects that improve the texture of food products, but carrageenan has no nutritional value. The same potent chemical effects that change the texture of processed foods can lead to harmful biological effects in human cells and in animals exposed to carrageenan. Carrageenan has been used in thousands of biological experiments over several decades, because it predictably causes inflammation. Inflammation is well-known to be the basis for many human diseases and is associated with over 100 human diseases, including inflammatory bowel disease, rheumatoid arthritis, and arteriosclerosis, and inflammation is also linked to cancer.

In experiments with human colonic cells in my laboratory, we have used small amounts of high molecular weight (undegraded or food grade) carrageenan and have determined the specific molecular mechanisms by which carrageenan causes inflammation. There are three major pathways by which carrageenan causes inflammation, including stimulation of an innate immune pathway. This pathway is also activated by pathogenic bacteria, such as Salmonella, and is stimulated due to the unusual chemical structure of carrageenan. Stimulation of this pathway is no accident; it is a direct result of the unusual chemical structure of carrageenan, and stimulation of this pathway has features that can lead to prolonged inflammatory effects. Also, the effects of carrageenan-induced inflammation are not limited to the intestine, and when

laboratory mice are exposed to low concentrations of carrageenan in their water for 18 days, they develop profound glucose intolerance and impaired insulin action. These responses are precursors of diabetes, which is associated with activation of the innate immune pathway that carrageenan stimulates.

In the past, many investigators used carrageenan to cause inflammation to study how specific drugs could reduce inflammation and to study the cells and cell products involved in the inflammatory response. Our recent work has looked instead within cells to study the signaling processes caused by carrageenan exposure, and we have found convincing evidence that carrageenan activates biologically significant pathways. Some of the effects of carrageenan are related to 1) activation of reactive oxygen species, 2) reduction in activity of sulfatase enzymes, and 3) activation of genes involved in carcinogenesis.

The work in my laboratory has used high molecular weight carrageenan in almost all of our experiments. In experiments with human colonic cells and tissue, we have used a concentration of carrageenan of 1 ug/ml. If an individual consumes 250 mg/day of carrageenan, the average intake cited in Encyclopedia Britannica, that represents 250 mg in an intestinal contents of about 5 liters [250 mg / 5L = 250 mg / 5000 ml = 1 mg / 20 ml = 1000 µg / 20 ml = 50 µg / ml], or about 50 times the concentration used in the laboratory work.

In the mouse experiments, we have used a concentration of carrageenan of 10 µg / ml in their water. The mice weigh about 25 grams and consume about 5 ml of water daily [10 µg / ml x 5 ml → 50 µg / 25 grams body weight = 2 µg / gram = 2 mg / kg], so have a daily intake of carrageenan of about 2 mg / kg, compared to a person who consumes [250 mg / 60 kg body weight = 4.25 mg / kg] over 4 mg / kg. Hence, the intake for the mice in these experiments is less than the anticipated daily intake for an adult in the United States. We note that some individuals consume less carrageenan, and some more than this amount, even up to several grams per day, depending on food choices. Please recall that 18 days of carrageenan intake in the mice produced profound glucose intolerance and impaired response to insulin, rather than intake over an entire lifetime during which humans are likely to ingest carrageenan.

When carrageenan was placed on the GRAS list in the 1950s, it was with the understanding that undegraded carrageenan did not contain degraded carrageenan. However, in addition to the clear harmful effects that arise from exposure to the higher molecular weight carrageenan, it is also clear at this point in time, that degraded carrageenan inevitably arises from higher molecular weight carrageenan. As the carrageenan manufacturers have shown in their Round Robin analysis of degraded carrageenan content in food-grade carrageenan (Marinalg Working Group report of January 2006 “Technical position on measurements related to meeting the EC molecular weight distribution specification for carrageenan and PES), up to 25% carrageenan of molecular weight less than 50,000 was measured in the food-grade carrageenan that was tested. Other research has indicated that acid digestion, heating, bacterial action, and mechanical processing can increase the amount of degraded carrageenan obtained from higher molecular weight carrageenan. Thus, it is not reasonable to evaluate and set a separate standard for undegraded and degraded

carrageenan, since they inevitably co-exist in food products. According to 7 CFR 205.600(a)(3): "the substance, itself, or its breakdown products do not have an adverse effect on human health …", and carrageenan does not meet this standard.

Reconsideration of the status of carrageenan in food products offers an opportunity to improve the safety of organic foods, and the health today and in the future of millions of Americans and other populations, as well. I urge you to act now and to really make a difference in the safety of organic foods in the United States. Carrageenan exposure clearly causes inflammation; the amount of carrageenan in food products is sufficient to cause inflammation; and degraded carrageenan and undegraded carrageenan are both harmful and can not be separately considered.

As an attachment, I am including a manuscript entitled “Review of Harmful Gastrointestinal Effects of Carrageenan in Animal Experiments” that may be helpful in these deliberations. I thank you for the opportunity to address this important issue.

Sincerely,

Joanne K. Tobacman, M.D.
Associate Professor of Clinical Medicine

Review of Harmful Gastrointestinal Effects of Carrageenan in Animal Experiments

Joanne K. Tobacman

College of Medicine, University of Iowa, Iowa City, Iowa, USA

In this article I review the association between exposure to carrageenan and the occurrence of colonic ulcerations and gastrointestinal neoplasms in animal models. Although the International Agency for Research on Cancer in 1982 identified sufficient evidence for the carcinogenicity of degraded carrageenan in animals to regard it as posing a carcinogenic risk to humans, carrageenan is still used widely as a thickener, stabilizer, and texturizer in a variety of processed foods prevalent in the Western diet. I reviewed experimental data pertaining to carrageenan's effects with particular attention to the occurrence of ulcerations and neoplasms in association with exposure to carrageenan. In addition, I reviewed from established sources mechanisms for production of degraded carrageenan from undegraded or native carrageenan and data with regard to carrageenan intake. Review of these data demonstrated that exposure to undegraded as well as to degraded carrageenan was associated with the occurrence of intestinal ulcerations and neoplasms. This association may be attributed to contamination of undegraded carrageenan by components of low molecular weight, spontaneous metabolism of undegraded carrageenan by acid hydrolysis under conditions of normal digestion, or the interactions with intestinal bacteria. Although in 1972, the U.S. Food and Drug Administration considered restricting dietary carrageenan to an average molecular weight > 100,000, this resolution did not prevail, and no subsequent regulation has restricted use. Because of the acknowledged carcinogenic properties of degraded carrageenan in animal models and the cancer-promoting effects of undegraded carrageenan in experimental models, the widespread use of carrageenan in the Western diet should be reconsidered. *Key words*: carcinogenesis, carrageenan, carrageenase, diet, furcelleran (furcellaran), hydrolysis, inflammatory bowel disease, nutrition, poligeenan, promoter, sulfated polysaccharide. *Environ Health Perspect* 109:983–994 (2001). [Online 24 September 2001]
http://ehpnet1.niehs.nih.gov/docs/2001/109p983-994tobacman/abstract.html

During the latter half of the twentieth century, inflammatory bowel disease and gastrointestinal malignancy have been major causes of morbidity and mortality in the United States. Even with improvements in treatment and cancer screening, colorectal cancer remains the second leading cause of cancer mortality in the United States. The Western diet has been considered a possible source of inflammatory bowel disease and colorectal malignancy, and intensive efforts have been undertaken to study the impact of specific constituents of the Western diet, such as fiber and fat (*1–3*).

One food additive, carrageenan, has been associated with induction and promotion of intestinal neoplasms and ulcerations in numerous animal experiments; however, carrageenan remains a widely used food additive. In 1982, the International Agency for Research on Cancer (IARC) (*4*) designated degraded carrageenan as Group 2B, noting sufficient evidence for the carcinogenicity of degraded carrageenan in animal models to infer that "in the absence of adequate data on humans, it is reasonable, for practical purposes, to regard chemicals for which there is sufficient evidence of carcinogenicity in animals as if they presented a carcinogenic risk to humans" (p. 90). The National Research Council has noted this designation for degraded carrageenan in their 1996 monograph (*5*). Recognizing the impact of carrageenan in animal models, several European and British investigators have advised against the continued use of carrageenan in food (*6–11*). Several reports have called attention to the problems associated with carrageenan consumption (*6–11*).

Extracted from red seaweed, carrageenan has been used in food products for centuries and was patented as a food additive for use in the United States in the 1930s. It has been used widely as a food additive, contributing to the texture of a variety of processed foods. It has also been used as a laxative, as treatment for peptic ulcer disease, and as a component of pharmaceuticals, toothpaste, aerosol sprays, and other products (*12–15*). In 1959, carrageenan was granted GRAS (Generally Regarded as Safe) status in the United States. GRAS substances are permitted to be incorporated into food products as long as good manufacturing processes are used and the substance is used only in sufficient quantity to achieve the desired effect (*16,17*).

In the United States, the status of carrageenan was reconsidered by the Food and Drug Administration, and an amendment to the Code of Federal Regulations for the food additive carrageenan was proposed in 1972 (*18*). To diminish the public's exposure to degraded carrageenan, the amendment supported inclusion of an average molecular weight for carrageenan of 100,000 and a minimum viscosity of 5 centipoises (cps) under specified conditions. However, the actual regulation was not amended, although several publications indicated that it had been modified (*7,8,19–23*). In 1979, the proposal to include the average molecular weight requirement of 100,000 and the associated viscosity requirement in the Code of Federal Regulations was withdrawn. It was anticipated that a new rule-making proposal on carrageenan that would comprehensively address all food safety aspects of carrageenan and its salts would be published in about a year, but this has not been forthcoming (*24,25*). The proposal withdrawal referred to interim specifications for food-grade carrageenan using the Food Chemical Codex; these include a viscosity stipulation, but no average molecular weight requirement (*26*).

In the Food Chemicals Codex and supplements, carrageenan is described with attention to specific requirements for its identification and tests of its properties, including its sulfate content, heavy metal content, solubility in water, content of acid-insoluble matter, and viscosity [a 1.5% solution is to have viscosity ≥ 5 cps at 75°C] (*26,27*). Although the viscosity is stipulated, viscosity may not adequately protect food-grade carrageenan from contamination by the lower molecular weight degraded carrageenans that IARC has denoted as Group 2B. Because undegraded carrageenan may have molecular weight in the millions, the actual viscosities of commercial carrageenans range from about 5 to 800 cps when measured at 1.5% at 75°C (*14*). Native carrageenan has molecular weights of 1.5×10^6–2×10^7 (*28*); poligeenan or degraded carrageenan is described as having average molecular weight of 20,000–30,000 (*4*). The average molecular weight of poligeenan has been described elsewhere as 10,000–20,000, but extending up to 80,000 (*29*). Food-grade carrageenan has been

Address correspondence to J.K. Tobacman, Department of Internal Medicine, University of Iowa Health Care, 200 Hawkins Drive, Iowa City, Iowa 52242-1081, USA. Telephone: (319) 356-3702. Fax: (319) 356-3086. E-mail: joanne-tobacman@uiowa.edu

Received 17 January 2001; accepted 17 March 2001.

described as having average molecular weight of 200,000–400,000 (*29*), and elsewhere as having molecular weight of 100,000–800,000 (*19*). Furcelleran (or furcellaran), a degraded carrageenan of molecular weight 20,000–80,000, has a sulfate content of 8–19% (*12,17*). No viscosity or minimum average molecular weight was designated for furcelleran in the 1972 or 1979 Federal Register documents (*18,24*). In the Food Chemical Codex (fourth edition), a 1.5% solution of furcelleran at 75°C is described as having minimum viscosity of 5 cps (*27*).

Today, carrageenan is still included among the food additives designated GRAS in the Code of Federal Regulations. The stipulations for its use include the following: *a*) it is a sulfated polysaccharide, the dominant hexose units of which are galactose and anhydrogalactose; *b*) range of sulfate content is 20–40% on a dry-weight basis; *c*) the food additive is used or intended for use in the amount necessary for an emulsifier, stabilizer, or thickener in foods, except for those standardized foods that do not provide for such use; *d*) to assure safe use of the additive, the label and labeling of the additive shall bear the name of the additive, carrageenan. Also included are similar standards for carrageenan salts and for furcelleran and furcelleran salts (*30*). In 1999–2000, approved uses for carrageenan were extended to include additional incorporation into food and medicinal products, including both degraded and undegraded carrageenan in laxatives (*31–33*).

For use in experimental models, degraded carrageenan (poligeenan) is derived from carrageenan by acid hydrolysis, frequently by a method developed by Watt et al. (*34*). This method is expected to yield a degraded carrageenan of average molecular weight 20,000–30,000 (*35*). Experiments demonstrate that reaction conditions similar to those of normal digestion can lead to the formation of degraded carrageenan (*9–11*). In addition, experimental data have revealed the contamination of food-grade carrageenan by substantial amounts of degraded carrageenan (*10*). Also, some bacteria are known to hydrolyze carrageenan and form low molecular weight derivatives (*36–40*).

The sections that follow and the accompanying tables summarize many experimental observations with regard to the intestinal effects of carrageenan. In addition, I review possible mechanisms for production of degraded carrageenan from undegraded carrageenan under physiologic conditions, as well as evidence that provides a basis for the mechanism of carrageenan's effects and for the reconsideration of the safety of carrageenan in the human diet.

Characteristics of Carrageenan

Three forms of carrageenan predominate, known as kappa, iota, and lambda. All have similar D-galactose backbones (alternating α-1,3 to β-1,4 linkages), but they differ in degree of sulfation, extent of branching, solubility, cation binding, and ability to form gels under different conditions. λ-Carrageenan is the least branched and the least gel forming; it is readily soluble at cold temperatures, in contrast to κ- or ι-carrageenan. Table 1 presents some of the basic characteristics of κ, ι, and λ carrageenan (*4,12–15,20–22,31–33,41–44*)

In addition to food additive uses, carrageenan has been used in cosmetics, pesticides, and pharmaceuticals, as well as in toothpaste and room deodorizers. It has been used as a treatment of ulcers and as an emulsifier in mineral oil laxatives, liquid petrolatum, and cod liver oil. However, its predominant role has been in food preparations, in which it is used across a wide variety of food groups because of its ability to substitute for fat and its ability to combine easily with milk proteins to increase solubility and improve texture. Hence, it is used in low-calorie formulations of dietetic beverages, infant formula, processed low-fat meats, whipped cream, cottage cheese, ice cream, and yogurt, as well as in other products. From its original use several centuries ago as a thickener in Irish pudding and its incorporation into blancmange, the food additive use has extended widely and cuts across both low-fat and high-fat diets. It is often combined with other gums, such as locust bean gum, to improve the texture of foods (*12–14,22,41,42*).

In 1977, data obtained by the survey of industry on the use of food additives produced an estimate of daily carrageenan intake of 100 mg for individuals older than 2 years. The 1971 survey of industry had indicated that formula-fed infants in the first 5 months of life had an intake of 108 mg/day (*21,43*). Informatics, Inc., in a report prepared for the Food and Drug Administration, cited daily carrageenan consumption of 45 mg (*19*); this is similar to the reported intake of 50 mg/day of carrageenan in France (*45*). Nicklin and Miller (*20*) reported intake of 0–1.5 g/day, depending on choice of diet and total food consumed. Although the Food and Nutrition Board of the National Research Council of the National Academy of Sciences of the United States in 1971 initially estimated 367 mg/day for carrageenan intake for individuals older than 2 years in the United States, this was subsequently revised to 11 mg/day. The wide range of estimates may be attributed to inconsistencies in how industry has reported carrageenan production and consumption data, variation in processed food formulations with regard to extent of incorporation of carrageenan, and changes in use of carrageenan in nonfood products. Daily individual consumption of between 50 mg/day and 100 mg/day is consistent with total consumption in the United States of 7,700 metric tons, as estimated for 1997 (*46*).

The content of carrageenan in several commonly consumed food products is summarized

Table 1. Characteristics of carrageenan (*4,12–15,27,28,41–49*).

Chemical composition	Hydrocolloid composed of α-D-1,3 and β-D-1,4 galactose residues that are sulfated at up to 40% of the total weight. Strong negative charge over normal pH range. Associated with ammonium, calcium, magnesium, potassium, or sodium salts.
Solubility	λ is readily soluble in cold or hot aqueous solution; κ is soluble in hot solution; treatment of aqueous solution with potassium ion precipitates κ-carrageenan.
Gel formation	λ does not form gels; λ and ι form right-handed helices; potassium chloride promotes gel formation of κ; calcium ion promotes gel formation of ι.
Metabolism	Hydrolysis of glycosidic linkages at lower pH, especially pH ≤ 3.0; also desulfation by sulfatases.
Viscosity	Near logarithmic increase in viscosity with increasing concentration. Viscosity of food-grade carrageenan defined as not less than 5 cps at 75°C for a 1.5% solution; viscosity ranges from 5 to 800 cps for 1.5% solution at 75°C.
Source	Red algae; predominantly aqueous extraction from *Chondrus, Gigartina,* and various *Eucheuma* species.
Molecular weight	Discrepancies in definitions. Native carrageenan reported to have average molecular weight of $1.5 \times 10^6 - 2 \times 10^7$; food-grade carrageenan reported as 100,000–800,000 or 200,000–400,000. Degraded carrageenan (poligeenan) has average molecular weight of 20,000–30,000; furcellaran has average molecular weight 20,000–80,000.
Properties	λ and κ combine easily with milk proteins to improve solubility and texture; serve as thickening agent, emulsifier, stabilizer.
Synergistic effects	With locust bean gum, increase in gel strength. Other hydrocolloids may also affect gel strength and cohesiveness.
Concentration in food products	0.005–2.0% by weight.
Major uses	Milk products, processed meats, dietetic formulations, infant formula, toothpaste, cosmetics, skin preparations, pesticides, laxatives

in Table 2. Because manufacturing practices vary and change over time and the food formulae are proprietary, carrageenan content is indicated by a range (*12,13,47–49*). The content is expressed as the percent by weight of carrageenan used in the production of the food.

Experimental Results in Animal Models

Intestinal lesions after exposure to carrageenan in animal models. Table 3 summarizes the laboratory investigations that associate exposure to carrageenan with the occurrence of intestinal lesions (*50–93*). Several animals were tested, including guinea pig, rat, monkey, mouse, rabbit, and ferret. The guinea pig seemed most susceptible to ulceration and the rat most susceptible to malignancy. Many studies used exposure to carrageenan in a drinking fluid, at concentrations generally of 1%. Some were feeding studies, in which carrageenan was added to a solid diet. Some studies used gastric or duodenal intubation to ensure intake at a specified level; however, this method may have affected the way that carrageenan was metabolized by gastric acid (*74,82–84,91*). Feeding of carrageenan with milk may also have affected study results, because carrageenan binds tightly to milk proteins (caseins), affecting its metabolism (*12–15, 22,41,42,47*). The degraded carrageenan used in most of the experiments had molecular weight from 20,000 to 40,000. Several major findings in relation to neoplasia and ulceration were observed in these animal studies. All of these studies observed the effects of carrageenan in comparison to appropriate control animals.

In the footnote to Table 3, several subdivisions of the table are indicated with citation of the entries from the table. The subdivisions include: *a*) studies in which carrageenan alone induces abnormal proliferation or malignancy, *b*) studies in which carrageenan alone induces intestinal ulcerations, *c*) studies in which carrageenan appears to be a promoter of malignancy in association with another agent, *d*) studies using a rat model, *e*) studies using a guinea pig model, *f*) studies using degraded carrageenan, *g*) studies using undegraded carrageenan, *h*) studies indicating uptake of carrageenan into an extraintestinal site(s), *i*) studies indicating intestinal breakdown of carrageenan into lower molecular weight forms, and *j*) studies demonstrating ulcerations in rats using degraded carrageenan. In the table, the classification of the carrageenan used in the experiments as κ, λ, or ι is indicated when this information is clear from the original report.

Neoplasia. Wakabayashi and associates (*72*) demonstrated the appearance of colonic tumors in 32% of rats fed 10% degraded carrageenan in the diet for less than 24 months. The lesions included squamous cell carcinomas, adenocarcinomas, and adenomas. With exposure to 5% degraded carrageenan in drinking water, there was a 100% incidence of colonic metaplasia after 15 months. Metastatic squamous cell carcinoma was observed in retroperitoneal lymph nodes in this experiment. In addition, macrophages that had metachromatic staining consistent with carrageenan uptake were observed in liver and spleen.

Other studies have demonstrated the development of polypoidal lesions and marked, irreversible squamous metaplasia of the rectal mucosa, the extent of which was associated with duration and concentration of carrageenan exposure (*67,70*). Oohashi et al. (*67*) observed a 100% incidence of colorectal squamous metaplasia that progressed even after degraded carrageenan intake was discontinued in rats fed 10% degraded carrageenan for 2, 6, or 9 months and sacrificed at 18 months.

Fabian et al. (*84*) observed adenomatous and hyperplastic polyps as well as squamous metaplasia of the anorectal region and the distal colon in rats given 5% carrageenan as a drinking fluid. Similarly, Watt and Marcus (*90*) observed hyperplastic mucosal changes and polypoidal lesions in rabbits given carrageenan as drinking fluid for 6–12 weeks at a concentration of 0.1–5%. Focal and severe dysplasia of the mucosal epithelium was observed in rabbits after 28 months of 1% degraded carrageenan in their drinking fluid (*58*).

Promotion of neoplasia. Several studies demonstrated an increased occurrence of neoplasia in relation to exposure to undegraded or degraded carrageenan and associated exposure to a known carcinogen. Experimental data with undegraded carrageenan included enhanced incidence of colonic tumors in rats treated with azoxymethane (AOM) and nitrosomethylurea (NMU), when carrageenan was added to the diet. Groups of rats received control diet; control diet with 15% carrageenan; 15% carrageenan plus 10 injections of AOM given weekly; carrageenan plus NMU; NMU alone; and AOM alone. AOM or NMU with carrageenan led to 100% incidence of tumors, versus 57% with AOM alone and 69% with NMU alone ($p < 0.01$). Controls had 0%, and carrageenan alone led to an incidence of 7%. In addition, when undegraded carrageenan was combined with AOM, there was a 10-fold increase in the number of tumors per rat (*73*). (Figure 1)

Using undegraded carrageenan as a solid gel at concentration 2.5% for 100 days, Corpet et al. (*50*) found that after exposure to azoxymethane, there was promotion of aberrant crypt foci by 15% ($p = 0.019$). Exposure of rats to 6% undegraded carrageenan in the diet for 24 weeks, with 1,2-dimethylhydrazine (1,2-DMH) injections weekly, was associated with an increase in tumors from 40% to 75% and with the more frequent occurrence of larger and proximal tumors (*57*).

Degraded carrageenan in the diet of rats at a 10% concentration in association with exposure to 1,2-DMH weekly for 15 weeks was associated with an increase in small intestinal tumors from 20% to 50% and in colonic tumors from 45% to 60% (*64*). Iatropoulos et al. (*77*) found that in rats given 5% degraded carrageenan in the drinking water for less than 30 weeks in association with injections of 1,2-DMH weekly, there were increases in poorly differentiated adenocarcinomas and in tumors of the ascending and transverse colon, as well as increased proliferation of cells in the deep glandular areas.

Several investigators have measured the effect of carrageenan on thymidine incorporation and colonic cell proliferation. Wilcox et al. (*51*) observed a 5-fold increase in

Table 2. Range of content of carrageenan in some commonly consumed foods.

Food	Percent carrageenan (g/100 g food)
Bakery products	0.01–0.1
Chocolate milk	0.01–0.2
Cottage cheese	0.02–0.05
Frosting base mix	3–4
Ice cream, frozen custard, sherbets, etc.	0.01–0.05
Jams and jellies	0.5–1.2
Liquid coffee whitener	0.3
Pie filling	0.1–1.0
Pimento olive stuffing	2.0
Processed cheese	0.01–0.06
Processed meat or fish	0.2–1.0
Pudding (nondairy)	0.1–0.5
Relishes, pizza, barbecue sauces	0.2–0.5
Yogurt	0.2–0.5

Because manufacturing processes vary and there can be substitutions of one hydrocolloid for another, the content of carrageenan for any individual product may differ from these estimates. Unpublished manufacturers' data indicate that these content estimates for processed cheese, frozen dairy dessert, cottage cheese, and jams and jellies are significantly lower than current usage (*4,13,14,47*).

thymidine kinase activity in colon cells with 5% undegraded or 5% degraded carrageenan. There was an associated 35-fold increase in proliferating cells in the upper third of crypts with degraded carrageenan and an 8-fold increase with undegraded carrageenan (*51*). With 5% λ-undegraded carrageenan fed to rats for 4 weeks, Calvert and Reicks (*55*) observed a 4-fold increase in thymidine kinase activity in the distal 12 cm of the colon ($p < 0.001$). Fath et al. (*59*) observed a 2-fold increase in colonic epithelial cell proliferation,

Table 3. Experimental data related to intestinal effects of dietary carrageenan exposure.

Experiment							Effects			
Type of carrageenan, molecular weight	Animal	Dose %CG	Dose (g/kg bw/day)	Duration	Route	Additional exposure	Digestive/ systemic	Histopathologic changes	Neoplastic changes	Reference
1. Undegraded[a,d]	Rat	10	27.4	8 days, sacrificed at 30 days	Jelly	AOM injection (5 mg/kg ip)	Less weight gain with 2.5% CG		UCG jelly (10% × 8 days) did not initiate tumor.	(*50*)
Undegraded[a,d]	Rat	0.25 2.5	0.2 4	100 days 100 days	Liquid Solid gel	AOM injection (20 mg/kg ip) prior to CG			UCG solid gel promoted growth of aberrant crypt foci (+15%; $p = 0.019$).	
2. Undegraded ι, >100,000[a,b]	Rat	0.5, 1.5, 5		≤ 91 days	Diet			Epithelial cell loss, macrophage infiltration, loss of crypts.	5-Fold increase in thymidine kinase activity in colon cells with 5% UCG or DCG. 35-Fold increase in proliferating cells in upper third of crypt with DCG, 8-fold with UCG.	(*51*)
Degraded ι, 20,000[a,b]	Rat	0.5, 1.5, 5		≤ 91 days	Diet					
3. Degraded ι, 30,000[c]	Guinea pig	2, 2.5, 5		7 days	Drink	Eicosapentaenoic acid (300 mg/kg/day) at 14 days	2% led to FOB+ at 7 days; 5% led to 38% mortality	Cecal ulcerations; foamy macrophages; small epithelial ulcerations at 2 days.		(*52*)
4. Degraded, 20,000–30,000[c]	Guinea pig	3	5.8	2–3 days	Drink			Microscopic mucosal changes from cecum to rectum; apparent macromolecule absorption by colonic epithelium, macrophage infiltration, macrophages with vacuoles.		(*35*)
5. Degraded[c]	Guinea pig	1, 2, 3	2, 3, 4	2 weeks	Drink			100% had cecal ulceration after 3% for 4 days; crypt abscesses.		(*53*)
6. Degraded[c]	Rat ileal cell monolayers		0–1.5 g/L	19, 30, 54 hr	Media				Retarded cell growth caused cell death; at 0.25g/L inhibited DNA synthesis by 20%.	(*54*)
7. Undegraded λ, 300,000[a,b]	Rat	5		4 weeks	Diet			3/8 had slight congestion and erythema of distal colon.	4-Fold increase in thymidine kinase activity in distal 12 cm of colonic mucosa.	(*55*)
8. Degraded ι[c]	Rat, Guinea pig	5 5		4 months 3 weeks	Drink Drink			Increased permeability to (^{3}H) PEG-900; ulcerations in guinea pig, crypt abscesses, macrophage infiltration.		(*56*)
9. Undegraded κ[a,d]	Rat	6	0.8	24 weeks	diet	1,2-DMH (20 mg/kg bw) SC × 16 wks			More tumors with UCG than control diet (75% vs. 40%); also larger, more proximal tumors.	(*57*)
10. Degraded λ[a,b,c]	Rabbit	1		8 weeks, 12 months, 28 months	Drink			Ulcerative lesions at 8 wks; at 12 months had chronic inflammatory changes.	At 28 months, focal and severe glandular atypism; precancerous changes seen.	(*58*)
11. Degraded[a,b,c]	Mice	10		10 days	Drink		Bloody diarrhea	Ulceration in proximal and distal colon, with dilatation of cecum and ascending colon.	2-Fold increase in colonic epithelial cell proliferation; increase in labeling indices and extension of proliferative compartment to upper third of crypt.	(*59*)
12. Degraded, (20,000–40,000), and Undegraded[a]	Cultured rat hepatocytes or intestinal mucosal cells		1 mg/10 mL or 1 mg/100 mL	20 hr 2 hr					DCG and UCG nonmutagenic in *Salmonella* mutagenicity test; DCG nongenotoxic by DNA repair test.	(*60*)
13. Undegraded κ, λ, and ι[e]	Rat	0.5	0.15–0.25	90 days	Drink			CG able to penetrate intestine; CG in mesenteric lymph node, and macrophages of villus and lamina propria.		(*61*)
14. Degraded[c]	Guinea pig	5		5 days	Drink			Small, superficial ulcerations over mucosal surface of cecum (1–111 ulcerations/cm^2).		(*62*)
15. Degraded[c]	Guinea pig	5		14 days	Drink	With ileotransversostomy		Ulcerations in cecum and proximal colon in unoperated. Postprocedure, crypt abscesses in rectum and ulcerations in distal colon and rectum. Macrophage infiltration.		(*63*)

Continued, next page

with increase in labeling indices in both proximal and distal colon and extensive increase of the proliferative compartment in the proximal colon to the upper third of the intestinal crypt, after exposure of mice to 10% degraded carrageenan in drinking water for 10 days.

Ulceration. Many studies have demonstrated significant ulceration of the cecum and/or large intestine after oral exposure to carrageenan in guinea pigs, rabbits, mice, rats, and rhesus monkeys (*34,35,53,56,58,59,62,63,65,68,70,71,75,*

Table 3. *Continued*

Experiment							Effects			
Type of carrageenan, molecular weight	Animal	%CG	Dose (g/kg bw/day)	Duration	Route	Additional exposure	Digestive/ systemic	Histopathologic changes	Neoplastic changes	Reference
16. Degraded[a,d]	Rat	10		2 weeks	Diet	1,2-DMH weekly injections for 15 weeks (10 mg/kg bw) with DCG vs. DMH alone			Increase in tumors of small intestine (50% vs. 25%) and colon (60% vs. 45%) with CG than occurred with DMH alone.	(*64*)
17. Degraded, (20,000–40,000)[a,b,c,g]	Rat	10	15	< 63 days	Diet	Germ-free vs. conventional gut flora		Erosions; aggregates of foamy metachromatic macrophages in submucosa and lamina propria.	Squamous metaplasia from anorectal junction to distal colon.	(*65*)
18. Degraded κ, λ, ι	Guinea pig	3		3 weeks	Drink		Diarrhea after 7 days. Moribund after 9 days of ιDCG.	No cecal or colonic lesions seen.		(*66*)
19. Degraded (20,000–40,000)[a,b,e]	Rat	10		2, 6, 9 months sacrificed at 18 months	Diet	Basal diet after DCG exposure		CG in mucosa and RE system.	100% incidence of colorectal squamous metaplasia that progressed after DCG intake discontinued.	(*67*)
20. Degraded[c]	Guinea pig	5		≤ 28 days	Drink			Cecal lesions after 24 hr; confluent ulcerations after 7 days. Macrophage infiltration.		(*68*)
21. Undegraded, (800,000) (largely κ)	Rat, hamster	0.5, 2.5, 5	0.36, 2, 4	Lifetime	Diet		Diarrhea in some	No difference in ulcerations from control.	Increased incidence of benign mammary tumors and testicular neoplasms (at 2.5% level) in rats only.	(*69*)
22. Degraded[a,b,c,g]	Rat	10		1 day to 12 weeks, some sacrificed at 27 weeks	Diet			Superficial erosions at anorectal junction at 24 hr; at 2 weeks, more proximal erosions.	Squamous metaplasia of rectal mucosa at 2 weeks; extended after no longer being fed CG.	(*70*)
23. Degraded[c]	Guinea pig	2		2 weeks	Drink		Loose stools by 2 weeks	100% with colonic ulcerations; 75% had over 200 ulcers.		(*34*)
24. Degraded[c]	Guinea pig	5		21 days			17/22 died by day 21	All had mucosal ulcerations from cecum to rectum by day 14.		(*71*)
25. Degraded (20,000–40,000)[a,b]	Rat	10, 5, 1 5 1 or 5		≤ 24 months 15 months 15 months	Diet drink Stomach tube				Squamous cell carcinomas, adenocarcinomas, adenomas. 32% and fed 10% diet had tumors. 100% incidence of metaplasia with 5% drink.	(*72*)
26.Undegraced λ[a,d]	Rat	15		≤ 40 weeks	Diet	NMU 2 mg twice weekly rectally for 3 weeks; AOM (8 mg/kg bw) SC for 10 weeks; UCG with NMU, UCG with AOM			100% had tumors with AOM and UCG vs. 57% with AOM alone. 100% had tumors with NMU and UCG vs. 69% with NMU alone. 0 tumors in control, 7% tumors with UCG alone. UCG with AOM had 10-fold increase in number of tumors per rat.	(*73*)
27. ι, (8,700–145,000)[e,f]	Rat		0.5	9 months	Gavage			Av MW of CG in liver was at 10,000; all CG in feces had MW < 100,000.		(*74*)
Undegraded κ/λ, (186,000–214,000)	Rat	5		13 weeks	Diet					
ι, (5,000–145,000)	Guinea pig	2		7–10 weeks	Diet					
ι, (5,000–145,000)	Guinea pig	1		2–3 weeks	Drink					
κ, 8,500–275,000	Guinea pig	1		2–3 weeks	Drink					
λ, (8,500–275,000)	Guinea pig	1		2–3 weeks	Drink					
Undegradec κ/λ, (185,000)	Rhesus monkey		0.05, 0.2, 0.5		Stomach tube					
ι			0.2		Stomach tube					

Continued, next page

78–80,82,83,86–93). Ulcerations arose in association with exposure to either degraded or undegraded carrageenan. Lesions occurred initially in the cecum of guinea pigs and rabbits, but could be induced in more distal parts of the colon of the guinea pig, as in an experiment in which carrageenan was introduced directly into the colon after ileotransversostomy (*63*). In rats, the ulcerative lesions appeared initially in distal colon and rectum (*8*). Undegraded and degraded carrageenan have been associated

Table 3. *Continued*

Experiment							Effects			
Type of carrageenan, molecular weight	Animal	%CG	Dose (g/kg bw/day)	Duration	Route	Additional exposure	Digestive/ systemic	Histopathologic changes	Neoplastic changes	Reference
28.κ[c] (314,000) (51,500) (8,500) λ, (275,000) (74,800) (20,800) ι, (145,000) (107,000) (88,000) (39,000) (21,000) (8,700) (5,000)	Guinea pig	1		2 weeks 2 weeks 2 weeks 2 weeks 2 weeks 2 weeks 2 weeks 10 weeks 2 weeks 10 weeks 2 weeks 2 weeks 10 weeks 2 weeks 10 weeks 2 weeks	Drink Drink Drink Drink Drink Drink Drink Diet Drink Diet Drink Drink Diet Drink Diet Drink			Cecal ulceration not seen with κ or λ; ι fractions of MW 21,000–107,000 led to ulcerations of cecum, crypt abscesses, and epithelial thinning. ι fractions absorbed and seen in vacuolated macrophages. Intense lysosomal enzymatic activity in macrophages of lamina propria.		(*75*)
29. Degraded ι (C16), (20,000)	Rhesus monkey	2		10 weeks	Drink			Macrophages given DCG had fibrillar material and vacuolations.		(*76*)
Undegraded κλ mixture, (800,000)	Rhesus monkey	1		10 weeks	Drink			Vacuolations seen with UCG.		
30. Degraded (ι, C16), (10,000–30,000)[a,d]	Rat	5	7.5 g, then 5 g	≤ 30 weeks	Drink, diet	1,2-DMH (20 mg/kg) SC/wk	Watery, bloody stools	Distal rectum transformed to stratified squamous by DMH with DCG.	DMH with DCG-induced proliferation of deep glandular areas; more poorly differentiated adenocarcinomas; more frequently found tumors of ascending and transverse colon with DMH and DCG.	(*77*)
31. Degraded[c,e]	Rat	0.2, 0.5, 5		≤ 12 weeks	Drink		Severe diarrhea in 3 days with 5%	DCG contained within macrophages of spleen, liver, kidney, small and large intestine; cecal and colonic ulcerations at 4 weeks.		(*78*)
	Guinea pig	0.25, 0.5		≤ 4 weeks	Drink		Diarrhea			
32. Degraded (ι, C16)[c]	Guinea pig	2, 0.2, 0.02		12 months, 10 months	Drink			2% CG in water, but not in milk, led to cecal ulceration in guinea pig. DCG in macrophages of submucosal layer in guinea pigs, rats, and monkeys. No cecal ulceration seen in rats or monkeys.		(*79*)
	Guinea pig	2		3 months	In milk					
	Rat	5		3 months	Drink					
	Monkey	2		10 weeks	Drink					
33. Degraded[c]	Guinea pig	2, 5	1.7–3.3	30–44 days	Drink	Trimethoprim/ Sulfamethoxazole	Blood in stools	Cecum and distal colon had ulcerations, crypt abscesses; enlarged cecal or colonic lymph nodes; more extensive ulceration with 5%; fewer lesions with antibiotic. Infiltration of foamy macrophages.		(*80*)
34. Undegraded κ, (200,000)	Pig		0.05, 0.2, 0.5	83 days	Jelly			Focal irregularities without ulcerations; thickened lamina propria; macrophage infiltration.		(*81*)
35. Degraded (C16, ι),[c] (20,000)	Rhesus monkey	0.5–2	0.7, 1.4, 2.9	7–14 weeks, then recovery for 20–24 weeks for some before sacrificed	Drink		Diarrhea, hemorrhage	Ulcerations of colon; hypertrophy of mesenteric lymph nodes and granulomas; multiple crypt abscesses; dose effect present.		(*82*)
Undegraded (largely κ), (800,000)	Rhesus monkey	1	1.3	7–14 weeks, then 11 weeks recovery	Drink			Without colonic changes.		
		1–3	0.05–1.25	≤ 12 weeks, after recovery	Tube					

Continued next page

with epithelial cell loss and erosions in rats (*51,65,70,87,93*).

Watt et al. (*34*) first observed ulcerations in response to carrageenan exposure in animal models more than three decades ago. They noted that 100% of guinea pigs given 2% degraded carrageenan as liquid for 20–30 days had colonic ulcerations and that 75% of the animals > 200 ulcers (*34*). When guinea pigs were given 1% undegraded carrageenan as liquid for 20–30 days, 80% developed colonic ulcerations (*92*). The

Table 3. *Continued*

Experiment							Effects			
Type of carrageenan, molecular weight	Animal	%CG	Dose (g/kg bw/day)	Duration	Route	Additional exposure	Digestive/ systemic	Histopathologic changes	Neoplastic changes	Reference
36. Undegraded	Guinea pig	5		1–45 days	Diet	Neomycin (0.1%) added	Diarrhea, hemorrhage	Multiple pinpoint cecal and colonic ulcerations after 3–5 weeks in guinea pig and rabbit. Macrophages increased; inclusions and vacuoles in macrophages; granulomas seen. Neomycin did not affect incidence of ulcers or time of onset.		(*83*)
	Rabbit	5			Diet					
Degraded[c]	Guinea pig	1, 2, 5		1–45 days	Drink					
	Rabbit	2			Drink					
Degraded	Humans		5-g dose	10 days	Diet			Patients had colon malignancy with colectomy planned to follow CG exposure, no ulcerations seen.		
	Ferret		1.5	28 days	Tube			No lesions seen.		
	Squirrel monkey		1.5	28 days	Tube			No lesions seen.		
	Rabbit, mouse		1.5	28 days	Tube			No lesions seen.		
	Rat	1		56 days	Drink		SI diarrhea			
	Rat	5					Diarrhea			
Undegraded	Rat	5		56 days	Diet		SI diarrhea			
	Hamster	5		6 months	Diet		Diarrhea			
37. Degraded (c16, ι), (20,000–30,000)[a,b,e]	Rat	5	6–10 0.5–5.0	≥ 25 weeks 1–15 months	Drink Tube		FOB+ by 3–7 days with > 5 g/kg/day; gross blood by 2–3 weeks	Metachromatic material thought to be CG found in RE cells of liver, spleen, lymph nodes, macrophages of lamina propria and submucosa. No cecal lesions.	Adenomatous and hyperplastic polyps in one rat. Squamous metaplasia of anorectal region and distal colon.	(*84*)
38. Undegraded (κ:λ = 70:30), (800,000)[e]	Rhesus monkey	1		7–12 weeks	Drink			No changes in liver.		(*85*)
Degraded (C16, ι), (20,000–30,000)[e]	Rhesus monkey	0.5, 1, 2		7–12 weeks	Drink			Membrane-bound vacuoles with fibrillar material in RE cells of liver.		
39. Degraded[c]	Guinea pig	5	≤ 2 g	20–45 days	Drink		FOB+, diarrhea by 1 week	Multiple ulcers in cecum, colon, and rectum in 100% of animals by day 30.		(*86*)
40. Degraded[a,b,c,g]	Rat	5		6 months	Drink			Ulceration of cecum in 4/12, associated with stricture; marked glandular hyperplasia at ulcer margins.		(*87*)
41. Undegraded[c]	Guinea pig	5		2–4 weeks	Diet			Ulceration of mucosa as consequence of macrophage accumulation in lamina propria, then submucosa.		(*88*)
Degraded[c]	Guinea pig	1		2–4 weeks	Drink					
42. Degraded[a,b,c]	Rabbit	0.1, 1, 5	0.07, 0.8, 1.4	6–12 weeks	Drink		Diarrhea, blood by day 7, weight loss	Ulceration of colon in 100% of those fed 1%; 60% of those fed 0.1%.	Hyperplastic mucosal changes, polypoidal lesions.	(*89,90*)
43. Degraded[c]	Guinea pig		4–5		Drink			Mucosal erosions in cecum, rarely into colon in guinea pig; without erosion in rat or mouse.		(*91*)
Degraded	Rat		≤ 16.5		Drink					
Degraded, Undegraded	Rat, mouse		0.07–4	28 days–6 months	Tube					
44. Undegraded	Guinea pig	1	≤ 1.5	20–30 days	Drink		FOB+	Multiple ulcerations of cecum; 80% had ulcerations. Crypt abscesses present; macrophages, with metachromatic material.		(*92*)
Degraded	Guinea pig	≤ 5	≤ 2	20–30 days	Drink		Diarrhea by 10 days, FOB+	100% had ulcerations; ulceration extended into distal colon and rectum.		
45. Degraded	Guinea pig	0.1–5		30 days–1 year	Drink		Weight loss in guinea pig and rabbit, not rat or mouse. Blood and mucous in stool.	Hemorrhagic and ulcerative lesions in cecum, colon, or rectum in all four species; crypt abscesses present.		(*93*)
	Rabbit				Drink					
	Rat				Drink					
	Mouse				Drink					

Abbreviations: AOM, azoxymethane; bw, body weight, CG,carrageenan; DCG, degraded carrageenan; DMH, dimethylhydrazine; FOB, fecal occult blood; ip, intraperitoneal; NMU, nitrosomethylurea; PEG, polyethylene glycol; SC, subcutaneous; SI, slight; tube, gastric intubation; UCG, undegraded carrageenan.

[a]Studies are associated with neoplastic changes, unlike studies predominantly demonstrating intestinal ulcerations. [b]Increased proliferation or neoplasm and carrageenan alone. [c]Ulcerations and carrageenan alone. [d]Neoplasms in which carrageenan promoted carcinogenesis. [e]Studies with uptake to lymph node or other site. [f]Study demonstrating breakdown to lower molecular weight. [g]Studies demonstrating ulcerations in rat using degraded carrageenan.

lesions were routinely produced with carrageenan concentrations of 0.1–1%, which is similar to the concentration in a variety of food products (*7,12–14*).

Grasso et al. (*83*) demonstrated pinpoint cecal and colonic ulcerations in guinea pigs and rabbits given 5% undegraded, as well as degraded, carrageenan in the diet for 3–5 weeks. Lesions were not observed in ferrets and squirrel monkeys given degraded carrageenan by gastric intubation (*83*). Other investigators have also observed ulcerations after exposure to either degraded or undegraded carrageenan (*75,88*). Engster and Abraham (*75*) observed ulceration of cecum in guinea pigs given ι-carrageenan of molecular weight 21,000–107,000, demonstrating ulcerations were also caused by higher molecular weight carrageenan. Cecal ulcerations were not found with exposures to κ or λ carrageenan of molecular weight varying from 8,500–314,000.

Investigators have noted that carrageenan-induced ulcerations of the colon are dose dependent and related to duration of exposure (*52,53,67,68,70,89,90*). Kitsukawa et al. (*52*) observed small epithelial ulcerations in guinea pigs who received carrageenan in their drinking fluid at two days. Olsen and Paulsen (*68*) observed cecal lesions after 24 hr and confluent ulcerations after 7 days in guinea pigs that ingested a 5% carrageenan solution. In rats, superficial erosions were observed at the anorectal junction at 24 hr after 10% dietary carrageenan (*70*); these extended more proximally over time. In 5 days of feeding with a 5% carrageenan solution, Jensen et al. (*62*) observed as many as 111 ulcerations/cm^2 over the mucosal surface of the cecum in the guinea pig.

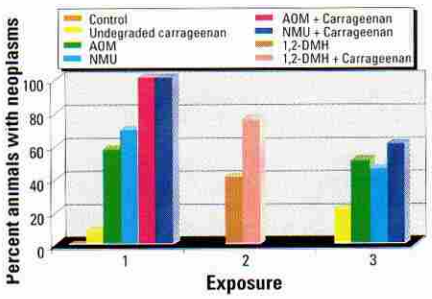

Figure 1. Carrageenan and promotion of neoplasms. No tumors were found in the control animals. With AOM and undegraded carrageenan, there was a 10-fold increase in the number of tumors per rat. See text for exposure regimens (*73*). 1,2-Dimethylhydrazine (1,2-DMH) alone caused neoplams in 40% of animals tested; with addition of undegraded carrageenan, 75% of exposed animals had tumors that were larger and occurred more frequently proximal (*57*). The combination of 1,2-DMH and degraded carrageenan was associated with an increase in small intestinal tumors from 20% to 50% of exposed animals and with an increase from 45% to 60% in large intestinal tumors (*64*).

Benitz et al. (*82*) observed a dose effect when degraded carrageenan was given at concentrations of 0.5–2% in drinking fluid to rhesus monkeys for 7–14 weeks. Watt and Marcus (*89*) observed that in rabbits given 0.1% degraded carrageenan as drinking fluid, 60% of the animals developed ulcerations, whereas 100% of those given 1% carrageenan had ulcerations when exposed for 6–12 weeks.

Resemblance to ulcerative colitis. Several investigators have noted the resemblance between the ulcerative lesions and accompanying inflammatory changes induced by carrageenan and the clinical spectrum of ulcerative colitis (*56,94–99*). Since the development of the carrageenan-induced model of ulcerative disease of the colon in 1969, carrageenan exposure has been used to model ulcerative colitis and to test for response to different treatments (*52,62,100,101*).

Clinical features in the experimental animals exposed to carrageenan have included weight loss, anemia, diarrhea, mucous in stools, and visible or occult blood in stools. The absence of small intestinal lesions and the lack of remission and exacerbation are also characteristic features of the carrageenan model (*99,102*).

Onderdonk (*94*) discussed the similarity between the carrageenan model of colitis and ulcerative colitis in humans and considered whether animal models for inflammatory bowel disease were also models for intestinal cancer because of the increased risk of colon cancer in individuals with ulcerative colitis. He reviewed the findings from carrageenan-treated animals, including loss of haustral folds, mucosal granularity, crypt abscesses, lymphocytic infiltration, capillary congestion, pseudopolyps, and strictures. Other observations have demonstrated an apparent sequence from colitis to squamous metaplasia and then to tumors of the colorectum (*67,72,102*). Atypical epithelial hyperplasia in the vicinity of carrageenan-induced ulcerations resembled findings from human ulcerative colitis that provide a link to intestinal neoplasia (*86,98*).

Proposed mechanism of development of lesions. A common feature observed in the animal models of ulceration in association with carrageenan exposure is macrophage

Table 4. Proposed mechanism for effects of carrageenan (*9,10,35,67,72,75,76,79,84–86,88,98,102,105,107–110,114,115*).

Site	Effect
Intestinal lumen	Ingested carrageenan can undergo acid hydrolysis in stomach, possible breakdown by intestinal bacteria.
Intestinal epithelial cells	Take up degraded carrageenan, as indicated by metachromatic staining from cecum to rectum. Vacuoles observed to contain metachromatic material. Epithelial cells may undergo lysis from effect of lysosomal disruption producing erosions.
Inflammatory infiltrate	Polymorphonuclear cells and macrophages infiltrate to site of intestinal inflammation. Macrophages have metachromatic staining associated with uptake of degraded carrageenan. Lysosomal vacuolation occurs as well as lysosomal disruption with release of intracellular enzymes from macrophage destruction, leading to intestinal ulcerations. Process of chronic inflammation, as with ulcerative colitis.
Macrophage circulation	Macrophages may circulate and may lead to extraintestinal effects related to carrageenan.

Table 5. Experimental evidence for presence of low molecular weight carrageenan in food-grade carrageenan and production of low molecular weight carrageenan by acid hydrolysis or by bacteria. (*9,10,36–40*).

Degraded carrageenan in food-grade carrageenan
25% of total carrageenans in eight food-grade κ-carrageenans had MW < 100,000
9% of total carrageenan in eight food-grade κ-carrageenans had MW < 50,000
Production of degraded carrageenan by acid hydrolysis of food-grade carrageenan
In simulated gastric fluid (including pepsin and HCL), κ-carrageenan at pH 1.2, 37°C
for 1 hr leads to 17% degraded carrageenan with MW < 20,000
for 2 hr leads to 25% with MW < 20,000
In simulated gastric fluid (including pepsin and HCL), κ-carrageenan at pH 1.9, 37°C
for 1 hr leads to 8% with MW < 20,000
for 2 hr leads to 10% with MW < 20,000
κ-carrageenan in solution at pH 1.0, 37°C, for 6 hours, leads to 25% with MW < 20,000
ι-carrageenan in solution at pH 1.0, 37°C, for 6 hours, leads to 10% with MW < 25,000
Hydrolysis of carrageenan by bacterial carrageenases
κ- and ι-carrageenase from cell-free supernatant from culture of *Cytophaga* genus
κ-carrageenase isolated from cell-free medium of cultured *Pseudomonas carrageenovora*
λ-carrageenase from cell-free medium of *Pseudomonas carrageenovora* cultures

MW, molecular weight.

infiltration (*35,56,63,65,68,75,76,78–81, 83,84,88,92,102–104*). Fibrillar material and metachromatic staining of the macrophages were observed. Notably, the macrophage lysosomes appeared to take up the fibrillar material and to become distorted and vacuolated. It appeared that colonic ulcerations developed as a result of macrophage lysosomal disruption, with release of intracellular enzymes, subsequent macrophage lysis, and release of intracellular contents that provoked epithelial ulceration (*75,76,79,84,85,88, 105,106*). In the rhesus monkey, Mankes and Abraham (*76*) observed vacuolated macrophages with fibrillar material when the animals were given undegraded carrageenan of molecular weight 800,000 as a 1% solution in their drinking fluid, demonstrating the occurrence of these changes after exposure to undegraded as well as to degraded carrageenan.

In an effort to clarify further the precise pathogenic changes that occurred, Marcus et al. (*35*) evaluated pre-ulcerative lesions after exposure of guinea pigs to degraded carrageenan for only 2–3 days. The animals received 3% drinking solution of carrageenan, with an average daily carrageenan intake of 5.8 g/kg. Early focal lesions were observed macroscopically in the cecum in only one animal with this brief exposure. However, in all test animals, a diffuse cellular infiltrate, with macrophages and polymorphonuclear leukocytes, was apparent microscopically. Inflammatory changes in the cecum and ascending colon were present in all animals, and in the distal colon and rectum in three of four animals. Metachromatic staining material was noted in the lamina propria of the colon and surface epithelial cells from cecum to rectum, as well as in colonic macrophages. The surface epithelial cells and the macrophages contained vacuoles filled with the metachromatic material, which was not found in the controls and not seen in more advanced lesions in previous studies. These early lesions suggested that the presence of degraded carrageenan within surface epithelial cells might be associated with the subsequent breakdown of the mucosa and to ulceration by a direct toxic effect on the epithelial cells (*35*).

Hence, a model of mechanical cellular destruction by disruption of lysosomes from carrageenan exposure arises from review of the experimental studies in animals. The observed changes in the lysosomes resemble the characteristic changes observed in some lysosomal storage diseases, in which there is accumulation of sulfated metabolites that cannot be processed further due to sulfatase enzyme deficiency (*107–110*). Table 4 presents a proposed mechanism of the effects of carrageenan.

Possible role of intestinal bacteria. The relationship between the intestinal microflora and the biologic activity of carrageenan has been reviewed (*111,112*). Investigators have examined the impact of antibiotics and alteration of the resident microbial flora on the activity of carrageenan. Grasso et al. (*83*) studied the impact of neomycin treatment on the development of ulcerations by carrageenan. Pretreatment against coliforms failed to attenuate the course of carrageenan-associated ulcerations (*80,83*). Pretreatment with metronidazole was effective in preventing carrageenan-induced colitis in another experiment, although there was no benefit in established colitis (*71*). Aminoglycosides administered after carrageenan exposure were associated with reduced mortality, but not with reduction in the number of colonic ulcerations (*94*). Hirono et al. (*65*) found increased ulcerations and squamous metaplasia from the anorectal junction to the distal colon in germ-free rats fed 10% carrageenan for less than 63 days.

Additional considerations about the mechanism of action of carrageenan involved the role of production of hydrogen sulfide gas from metabolism of carrageenan in the digestive tract. Because carrageenan is heavily sulfated (up to 40% by weight), bacterial sulfatases and sulfate reductases can produce hydrogen sulfide gas or HS^- from carrageenan. Carrageenan, as well as other sulfated polysaccharides, has been shown to stimulate H_2S production from fecal slurries (*113*). Sulfide has been implicated in the development of ulcerative colitis, perhaps attributable to interference with butyrate oxidation by colonic epithelial cells (*114,115*). Butyrate has been shown to induce intestinal cellular differentiation, suppress intestinal cell growth, and decrease expression of c-*myc*, among other functions in colonic epithelial cells (*116–118*).

No fermentation of carrageenan was reported after testing with 14 strains of intestinal bacteria. The increase in sulfide production observed arising from incubation of λ-carrageenan with colonic bacteria demonstrates that intestinal metabolism of carrageenan does occur. However, data pertaining to breakdown of carrageenan by fecal organisms are limited (*112,113*).

Extraintestinal manifestations of carrageenan exposure. Trace amounts of undegraded carrageenan have been reported to cross the intestinal barrier, with accumulation of label in intestinal lymph nodes (*61,74*). Several investigators have noted uptake of carrageenan by intestinal macrophages with subsequent migration of these macrophages to lymph nodes, spleen, and liver (*61,67,74,78,82,84,85*).

In association with carrageenan-induced intestinal ulcerations, Delahunty et al. (*56*) observed an increased permeability to large molecules, such as [^{3}H]PEG (polyethylene glycol)-900. This finding suggested that the intestinal changes induced by carrageenan may be a factor in subsequent absorption of carrageenan or other large molecules.

Other experimental data. Because it can induce acute inflammation, carrageenan has been widely used in experimental models of inflammation to assess activity of anti-inflammatory drugs and to study mediators of inflammation (*4,61,106,119,120*). Injected into an experimental site, such as the plantar surface of a rat's paw, pleural cavity, or subcutaneous air bleb, carrageenan induces an inflammatory response, with edema, migration of inflammatory cells, predominantly polymorphonuclear leukocytes, and possibly granuloma formation (*61,120*). Undegraded carrageenans *in vitro* can inhibit binding of basic fibroblast growth factor (bFGF), transforming growth factor β-1, and platelet-derived growth factor but not insulin-like growth factor-1 or transforming growth factor-α (*121*).

Macrophage injury and destruction caused by carrageenan may be a factor in the reduced cytotoxic lymphocytic response associated with carrageenan exposure *in vivo* (*122*). In addition to depression of cell-mediated immunity, impairment of complement activity and of humoral responses have been reported. Prolongation of graft survival and potentiation of tumor growth have been attributed to the cytopathic effect on macrophages (*96,123*). Because of its effect on T-cells, carrageenan has been studied for its impact on viral infections with herpes simplex virus types 1 and 2 (*124*) and HIV-1 (*125,126*), as well as infections with *Chlamydia trachomatis* (*127*).

In experimental systems, undegraded carrageenan has produced destruction of several different cell types in addition to macrophages, including small intestine epithelial cell monolayers (*54*), androgen-dependent malignant prostatic cells (*128*), bFGF-dependent endothelial cell line (*128*), rat mammary adenocarcinoma 13762 MAT cells (*129*), and human mammary myoepithelial cells (*130*). Lysosomal inclusions and vacuolation have been observed in macrophages, intestinal epithelial cells, and myoepithelial cells exposed to carrageenan (*79,85,131*).

Injections of carrageenan were noted to induce sarcomas, as well as mammary tumors in animal models, in an early study (*132*). In other experiments, mammary and testicular tumors have been observed (*69,133*). Carrageenan has also been noted to have anticoagulant activity, and large systemic doses have been fatal through nephrotoxicity (*4*).

Mechanisms for Production of Degraded Carrageenan from Undegraded Carrageenan

Gastrointestinal metabolism of carrageenan to form smaller molecular weight components has been observed by several investigators, who reported that carrageenan of high molecular weight changed during intestinal passage, compatible with hydrolysis yielding lower molecular weight components (*9,10,74,75*).

Under conditions such as might occur in digestion, 17% of food-grade carrageenan degraded to molecular weight < 20,000 in 1 hr at pH 1.2 at 37°C. At pH 1.9 for 2 hr at 37°C, 10% of the carrageenan had molecular weight less than 20,000 (*9*). These data suggest that substantial fractions of lower molecular weight carrageenan are likely to arise during normal digestion.

Table 5 presents data with regard to contamination of food-grade carrageenan by lower molecular weight carrageenan. Twenty-five percent of total carrageenans in eight food-grade carrageenans were found to have molecular weight < 100,000, with 9% having molecular weight < 50,000 (*9*). In addition, several bacteria have been identified that are able to hydrolyze carrageenan into smaller products, including tetracarrabiose. These bacteria, including *Cytophaga* species and *Pseudomonas carrageenovora*, are of marine origin; it is unknown whether the human microbial flora can perform similar hydrolysis reactions (*36–40,134*).

Extent of Human Exposure to Carrageenan

Indirect evidence relating exposure to carrageenan and the occurrence of ulcerative colitis and intestinal neoplasms consists of the similar geographic distribution between higher consumption of carrageenan and higher incidence of inflammatory bowel disease and colorectal cancer. Ulcerative colitis is more common in North America, the United Kingdom, and Scandinavia, and less common in Central and Southern Europe, Asia, and Africa (*135*). This incidence distribution is similar to distributions for colorectal malignancy and for carrageenan consumption, providing some ecologic evidence to support a potential etiologic role of carrageenan in human disease (*46,136*).

The reported TD_{50} (tumorigenic dose 50% = the dose rate, in milligrams per kilogram body weight per day, which will halve the probability of remaining tumorless over the life span of the exposed animal) by the Carcinogenic Potency Database for degraded carrageenan is 2,310 mg/kg body weight/day, based on rodent experiments (*137,138*). This extrapolates to 138.6 grams for a 60-kg individual. If the total carrageenan intake per person in the United States is about 100 mg a day (*43*), about 9 mg of carrageenan with molecular weight < 50,000 is likely to be ingested through contamination of food-grade carrageenan by degraded carrageenan, and at least 8 mg with molecular weight < 20,000 is likely to arise during normal digestion (simulated by exposure to pH 1.9 with pepsin for 1 hr at 37°C). This suggests an average intake of about 10 mg/day of degraded carrageenan for an individual older than 2 years of age in the United States.

An important issue is whether 10 mg/day degraded carrageenan is safe to ingest. By the Delaney clause, no carcinogen should be permitted in food. The Food Quality Protection Act (FQPA) established a usage level for negligible risk associated with pesticide residue in food at 1 ppm (*139,140*). Applying this standard to the extrapolated TD_{50} for degraded carrageenan for a 60-kg person, the anticipated average intake of 10 mg/day is 70-fold greater than this standard (138.6 g/10^6/day). These calculations do not take into consideration possible exposure to furcellaran (molecular weight 20,000–80,000), or the wide range of possible intakes of carrageenan.

Conclusion

Inflammatory bowel disease and colorectal malignancy represent major sources of morbidity and mortality in the United States. A possible factor in the etiology of these pathologies is exposure to carrageenan.

Several investigators have expressed their concerns about the use of undegraded carrageenan in food products (*6–10*), yet no legislative protection to restrict incorporation of low molecular weight fractions has been enacted. In fact, there has been no substantive review by the Food and Drug Administration of carrageenan since the studies undertaken more than two decades ago. However, there has been increased evidence regarding the cancer-promoting activity of undegraded carrageenan and further confirmation of the carcinogenic potential of degraded carrageenan.

Evidence for the role in carcinogenesis of carrageenan appears to support a nongenotoxic model based on direct toxic effects, for carrageenan has been nonmutagenic in *Salmonella* mutagenicity testing and nongenotoxic by DNA repair tests (*60,102*). A model of cellular destruction—from disruption of lysosomes by accumulation of carrageenan by-products or by interference with normal cellular oxidation–reduction processes from sulfate metabolites—emerges from review of the experimental studies. The impact of sulfatases, of either bacterial or human origin, on the metabolism of carrageenan requires further investigation. By interference with the normal intracellular feedback mechanisms associated with arylsulfatase activity, including steroid sulfatase, the highly sulfated carrageenan may have an impact on the availability of active, unsulfated hormones, such as dehydroepiandrosterone, derived from dehydroepiandrosterone-sulfate, and estrone-1, derived from estrone-1 sulfate.

Genetic characteristics that affect sulfatase and hydrolysis reactions as well as the individual intestinal microflora may influence how carrageenan is metabolized and how its effects are manifested. These factors may determine how carrageenan is metabolized differently by different individuals, but these characteristics may not be accessible to manipulation. A basic factor that can be controlled is the intake of carrageenan, which is amenable to dietary modification or food additive regulation.

Although carrageenan is widely used as a food additive for its texture-enhancing properties, other gums, some of which are used in combination with carrageenan, such as locust bean gum, gum arabic, alginate, guar gum, or xanthan gum, potentially can be used alone or in different combinations as substitutes for carrageenan (*41,46*). Alternatively, higher fat composition can lead to changes in food properties that may compensate for exclusion of carrageenan. Other hydrocolloids that are used as stabilizers and thickeners have not been associated with harmful gastrointestinal effects, and it is reasonable to expect that they could replace carrageenan in many food products. Although the dietary fibers pectin and psyllium affect intestinal motility, ulcerations or neoplasms have not been induced with either these or the other water-soluble polymers used as food additives. In contrast, other highly sulfated polysaccharides, amylopectin sulfate and dextran sulfate sodium, have induced ulcerations and neoplasia, suggesting that the degree of sulfation and polysaccharide molecular weight may be critical for induction of the observed effects (*102*).

The major pieces of evidence that support an argument to reconsider the advisability of use of carrageenan as a GRAS food additive are:

- Degraded carrageenan is a known carcinogen in animal models
- Undegraded carrageenan is a known co-carcinogen in animal models of carcinogenesis
- In animal models, both degraded and undegraded carrageenan have been associated with development of intestinal ulcerations that resemble ulcerative colitis
- Hydrolysis such as may occur by exposure to gastric acid in the human stomach can lead to the depolymerization of undegraded carrageenan and the availability of degraded carrageenan
- Food-grade carrageenan may be contaminated with low molecular weight, degraded

carrageenan that may arise during food processing

- The use of a viscosity measurement to characterize a carrageenan sample is insufficient because the presence of a small number of large molecules (and undegraded carrageenan may have molecular weight in the millions) may obscure a significant low molecular weight fraction.

The potential role of carrageenan in the development of gastrointestinal malignancy and inflammatory bowel disease requires careful reconsideration of the advisability of its continued use as a food additive.

References and Notes

1. Ries LAG, Kosary CL, Hankey BF, Miller BA, Clegg L, Edwards BK. SEER Cancer Statistics Review 1973–1996. Bethesda, MD:National Cancer Institute, 1999.
2. Schottenfeld D, Winawer SJ. Cancers of the large intestine. In: Cancer Epidemiology and Prevention (Schottenfeld D, Fraumeni J, eds). 2nd ed. New York:Oxford University Press, 1996;813–840.
3. Schatzkin A. Available: http://rex.nci.nih.gov/NCI_Pub_Interface/raterisk/risks129.html [cited 6 October 2000].
4. IARC. IARC Working Group on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Carrageenan. IARC Monogr Eval Carcinog Risk Hum 31:79–94 (1983).
5. National Research Council. Carcinogens and Anti-carcinogens in the Human Diet. Washington, DC:National Academy Press, 1996;398.
6. Marcus R, Watt J. Danger of carrageenan in foods and [Letter]. Lancet 1:338 (1981).
7. Marcus R, Watt J. Potential hazards of carrageenan [Letter]. Lancet 1:602–603 (1980).
8. Marcus R. Harmful effects of carrageenan fed to animals. Cancer Detect Prev 4:129–134 (1981).
9. Ekstrom L-G. Molecular weight distribution and the behavior of kappa-carrageenan on hydrolysis. Carbohydr Res 135:283–289 (1985).
10. Ekstrom L-G, Kuivinen J, Johansson G. Molecular weight distribution and hydrolysis behavior of carrageenans. Carbohydr Res 116:89–94 (1983).
11. Yu G, Ioanoviciu AS, Sikkander SA, Thanawiroon C, Toida T, Tobacman J, Linhardt RJ. Unpublished data.
12. Klose RE, Glicksman M. Gums. In: Handbook of Food Additives (Furia TE, ed). Cleveland, OH:The Chemical Rubber Co., 1968;313–375.
13. Towle GA. Carrageenan. In: Industrial Gums: Polysaccharides and Their Derivatives (Whistler RL, ed). New York:Academic Press, Inc., 1973;84–109.
14. Moirano AL. Sulfated seaweed polysaccharides. In: Food Colloids (Graham HD, ed). Westport, CT:AVI Publishing Co., 1977;347–381.
15. Daniel JR, Voragen ACJ, Pilnik W. Starch and other polysaccharides. In: Ullmann's Encyclopedia of Industrial Chemistry, Vol A 25 (Elvers B, Hawkins S, Russey W, eds). New York:VCH Verlagsgesellschaft, 1994;21–62.
16. Substances that are generally recognized as safe. Fed Reg 21:9368–9370.
17. Food and Drugs: Food Additives. 21 C.F.R. 121.101,121.1063,121.1066,121.1067,121.1069, 1969.
18. Proposed Revision of Food Additive Regulations and Deletion of Chondrus Extract (Carrageenin) from Generally Regarded as Safe (GRAS) List. 37 Fed Reg 15434.
19. Informatics, Inc. Carrageenan. Arlington, VA:National Technical and Information Service, 1972;1–68.
20. Nicklin S, Miller K. Intestinal uptake and immunological effects of carrageenan—current concepts. Food Addit Contam 6(4):425–436 (1989).
21. Food and Nutrition Board, National Research Council. Estimating Distribution of Daily Intakes of *Chondrus* Extract (Carrageenan): Committee on GRAS List Survey–Phase III. Appendix C. Washington, DC:National Academy of Sciences, 1976;1–7.
22. Stanicoff DJ, Renn DW. Physiological effects of carrageenan. In: ACS Symposium Series (15): Physiological Effects of Food Carbohydrates (Gould RF, ed). Washington, DC:American Chemical Society, 1975; 282–295.
23. Pintauro SJ, Gilbert SW. The effects of carrageenan on drug-metabolizing enzyme system activities in the guinea-pig. Food Chem Toxicol 28:807–811 (1990).
24. Carrageenan, Salts of Carrageenan and Chondrus Extract (Carrageenin); Withdrawal of Proposal and Termination of Rulemaking Proceeding. Fed Reg 44:40343–40345.
25. International Food Additives Council and FMC Corporation-Marine Colloids Division, filing of Food Additive Petitions; Hercules, Inc.; Notice of Receipt of Citizen Petition; Request for Comments. Fed Reg 57:49483–49485.
26. National Research Council. Food Chemical Codex. 2nd ed, suppl 2. Washington, DC:National Academy of Science, 1975.
27. National Research Council. Food Chemical Codex. 4th ed. Washington, DC:National Academy of Science, 1996.
28. Tong H-K, Lee K-H, Wong H-A. The molecular weight and viscosity of the water-soluble polysaccharide(s) from *Eucheuma spinosum*. Carbohydr Res 81:1–6 (1980).
29. Weiner ML. Toxicological properties of carrageenan. Agents Actions 32(1/2):46–51 (1991).
30. Food and Drugs: Food Additives Permitted for Direct Addition to Food for Human Consumption. 21 C.F.R. 172.620,172.626,172.655,172.660, 2000.
31. Food and Drugs: Substances Generally Regarded as Safe. 21 C.F.R. 182.7255, 1999.
32. Food and Drugs: New Drugs. 21 C.F.R. 310.545, 1999.
33. Food and Drugs: 21 C.F.R. 133.178, 133.179, 136.110, 139.121, 139.121, 139.122, 150.141, 150.161, 176.170 (2000).
34. Watt J, McLean C, Marcus R. Degradation of carrageenan for the experimental production of ulcers in the colon. J Pharm Pharmacol 31:645–646 (1979).
35. Marcus SN, Marcus AJ, Marcus R, Ewen SWB, Watt J. The pre-ulcerative phase of carrageenan-induced colonic ulceration in the guinea-pig. Int J Exp Pathol 73:515–526 (1992).
36. Sarwar G, Matoyoshi S, Oda H. Purification of a κ-carrageenase from marine *cytophaga* species. Microbiol Immunol 31:869–877 (1987).
37. Weigl J, Yaphe W. The enzymic hydrolysis of carrageenan by *pseudomonas carrageenovora*: purification of a κ-carrageenase. Can J Microbiol 12:939–947 (1986).
38. Potin P, Sanseau A, LeGall Y, Rochas C, Bloareg B. Purification and characterization of a new κ-carrageenase from a marine *cytophaga*-like bacterium. Eur J Biochem 201:241–247 (1991).
39. McLean MW, Williamson FB. κ-Carrageenase from *Pseudomonas carrageenovora*. Eur J Biochem 93:553–558 (1979).
40. Johnston KH, McCandless EL. Enzymic hydrolysis of the potassium chloride soluble fraction of carrageenan: properties of "lambda carrageenases" from *Pseudomonas carrageenovora*. Can J Microbiol 19(7):779–788 (1973).
41. Friedman LJ, Greenwald CG. Food additives. In: Encyclopedia of Chemical Technology, Vol 11 (Howe-Grant M, ed). 4th ed. New York:John Wiley & Sons, 1994;805–833.
42. Meer WA. Plant hydrocolloids. In: Food Colloids (Graham HD, ed). Westport, CT:AVI Publishing Company, Inc., 1977;522–539.
43. Food and Nutrition Board, National Research Council. The 1977 Survey of Industry on the Use of Food Additives: Committee on GRAS List Survey–Phase III. Part 3. PB 80–113418. Washington, DC:National Academy of Sciences, 1979.
44. Anderson W. Carrageenan: structure and biological activity. Can J Pharm Sci 2:81–90 (1967).
45. Comité "Additifs Alimentaires" du CNERNA. Toxicological evaluation of carrageenans. 10-Conclusions: acquired knowledges and problems requiring further researches. Sciences des aliments 4:429–438.
46. Will R, Zuanich J, DeBoo A, Ishikawa Y. Water-soluble polymers. Menlo Park, CA:Chemical Economics Handbook - SRI International, 1999;582.0000E–582.0003V.
47. Piculell L. Gelling carrageenans. In: Food Polysaccharides and Their Applications. New York:Marcel Dekker, Inc., 1995;205–244.
48. Food and Nutrition Board, National Research Council. 1977 Survey of Industry on the Use of Food Additives. Summarized Data: Committee on GRAS List Survey–Phase III. Washington, DC:National Academy of Sciences, 1979;978–987.
49. Food Protection Committee, Food and Nutrition Board, National Research Council. Chemicals Used in Food Processing. Publication 1274. Washington, DC:National Academy of Sciences, 1965;31–34.
50. Corpet DE, Taché S, Préclaire M. Carrageenan given as a jelly, does not initiate, but promotes the growth of aberrant crypt foci in the rat colon. Cancer Lett 114:53–55 (1997).
51. Wilcox DK, Higgins J, Bertram TA. Colonic epithelial cell proliferation in a rat model of nongenotoxin-induced colonic neoplasia. Lab Invest 67:405–411 (1992).
52. Kitsukawa Y, Saito H, Suzuki Y, Kasanuki J, Tamura Y, Yoshida S. Effect of ingestion of eicosapentaenoic acid ethyl ester on carrageenan-induced colitis in guinea pigs. Gastroenterology 102:1859–1866 (1992).
53. Marcus AJ, Marcus SN, Marcus R, Watt J. Rapid production of ulcerative disease of the colon in newly-weaned guinea-pigs by degraded carrageenan. J Pharm Pharmacol 41:423–426 (1989).
54. Ling K-Y, Bhalla D, Hollander D. Mechanisms of carrageenan injury of IEC18 small intestinal epithelial cell monolayers. Gastroenterology 95:1487–1495 (1988).
55. Calvert RJ, Reicks M. Alterations in colonic thymidine kinase enzyme activity induced by consumption of various dietary fibers. Proc Soc Exp Biol Med 189:45–51 (1988).
56. Delahunty T, Recher L, Hollander D. Intestinal permeability changes in rodents: a possible mechanism for degraded carrageenan-induced colitis. Food Chem Toxicol 25:113–118 (1987).
57. Arakawa S, Okumua M, Yamada S, Ito M, Tejima S. Enhancing effect of carrageenan on the induction of rat colonic tumors by 1,2-dimethylhydrazine and its relation to β-glucuronidase activities in feces and other tissues. J Nutr Sci Vitaminol (Tokyo) 32:481–485 (1986).
58. Kitano A, Matsumoto T, Hiki M, Hashimura H, Yoshiyasu K, Okawa K, Kuwajima S, Kobayashi K. Epithelial dysplasia of the rabbit colon induced by degraded carrageenan. Cancer Res 46:1374–1376 (1986).
59. Fath RB, Deschner EE, Winawer SJ, Dworkin BM. Degraded carrageenan-induced colitis in CF_1 mice. Digestion 29:197–203 (1984).
60. Mori H, Ohbayashi F, Hirono I, Shimada T, Williams GM. Absence of genotoxicity of the carcinogenic sulfated polysaccharide carrageenan and dextran sulfate in mammalian DNA repair and bacterial mutagenicity assays. Nutr Cancer 6:92–97 (1984).
61. Nicklin S, Miller K. Effect of orally administered food-grade carrageenans on antibody-mediated and cell-mediated immunity in the inbred rat. Food Chem Toxicol 22:615–621 (1984).
62. Jensen BH, Andersen JO, Poulsen SS, Olsen PS, Rasmussen SN, Hansen SH, Hvidberg DF. The prophylactic effect of 5-aminosalicylic acid and salazosulphapyridine on degraded-carrageenan-induced colitis in guinea pigs. Scand J Gastroenterol 19:299–303 (1984).
63. Olsen PS, Kirkegaard P, Poulsen SS. The effect of ileo-transversostomy on carrageenan-induced colitis in guinea pig. Scand J Gastroenterol 18:407–410 (1983).
64. Kawaura A, Shibata M, Togei K, Otsuka H. Effect of dietary degraded carrageenan on intestinal carcinogenesis in rats treated with 1,2-dimethylhydrazine dihydrochloride. Tokushima J Exp Med 29:125–129 (1982).
65. Hirono I, Sumi Y, Kuhara K, Miyakawa M. Effect of degraded carrageenan on the intestine in germfree rats. Toxicol Lett 8:207–212 (1981).
66. Norris AA, Lewis AJ, Zeitlin IJ. Inability of degraded carrageenan fractions to induce inflammatory bowel ulceration in the guinea pig. J Pharm Pharmacol 33:612–613 (1981).
67. Oohashi Y, Ishioka TT, Wakabayashi K, Kuwabara N. A study of carcinogenesis induced by degraded carrageenan arising from squamous metaplasia of the rat colorectum. Cancer Lett 14:267–272 (1981).
68. Olsen PS, Poulsen SS. Stereomicroscopic and histologic changes in the colon of guinea pigs fed degraded carrageenan. Acta Pathol Microbiol Scand Sect A 88:135–141 (1980).
69. Rustia M, Shubik P, Patil K. Lifespan carcinogenicity tests with native carrageenan in rats and hamsters. Cancer Lett 11:1–10 (1980).
70. Oohashi Y, Kitamura S, Wakabayashi K, Kuwabara N, Fukuda Y. Irreversibility of degraded carrageenan-induced colorectal squamous metaplasia in rats. Gann 70:391–392 (1979).

71. Onderdonk AB, Hermos JA, Dzink JL, Bartlett JG. Protective effect of metronidazole in experimental ulcerative colitis. Gastroenterology 74:521–526 (1978).
72. Wakabayashi K, Inagaki T, Fujimoto Y, Fukuda Y. Induction by degraded carrageenan of colorectal tumors in rats. Cancer Lett 4:171–176 (1978).
73. Watanabe K, Reddy BS, Wong CQ, Weisburger JH. Effect of dietary undegraded carrageenan on colon carcinogenesis in F344 rats treated with azoxymethane or methylnitrosourea. Cancer Res 38:4427–4430 (1978).
74. Pittman KA, Golberg L, Coulston F. Carrageenan: the effect of molecular weight and polymer type on its uptake, excretion and degradation in animals. Food Cosmet Toxicol 14:85–93 (1976).
75. Engster M, Abraham R. Cecal response to different molecular weights and types of carrageenan in the guinea pig. Toxicol Appl Pharmacol 38:265–282 (1976).
76. Mankes R, Abraham R. Lysosomal dysfunction in colonic submucosal macrophages of rhesus monkeys caused by degraded iota carrageenan. Proc Soc Exp Biol Med 150:166–170 (1975).
77. Iatropoulos MJ, Golberg L, Coulston L. Intestinal carcinogenesis in rats using 1,2-dimethylhydrazine with or without degraded carrageenan. Exp Mol Pathol 23:386–401 (1975).
78. Grasso P, Gangolli SD, Butterworth KR, Wright MG. Studies on degraded carrageenan in rats and guinea-pigs. Food Cosmet Toxicol 13:195–201 (1975).
79. Abraham R, Fabian RJ, Golberg MB, Coulston F. Role of lysosomes in carrageenan-induced cecal ulceration. Gastroenterology 67:1169–1181 (1974).
80. Van der Waaif D, Cohen BJ, Anver MR. Mitigation of experimental inflammatory bowel disease in guinea pigs by selective elimination of the aerobic gram-negative intestinal microflora. Gastroenterology 67:460–472 (1974).
81. Poulsen E. Short-term peroral toxicity of undegraded carrageenan in pigs. Food Cosmet Toxicol 11:219–227 (1973).
82. Benitz K-F, Golberg L, Coulston F. Intestinal effects of carrageenans in the rhesus monkey. Food Cosmet Toxicol 11:565–575 (1973).
83. Grasso P, Sharratt M, Carpanini FMB, Gangolli SD. Studies on carrageenan and large-bowel ulceration in mammals. Food Cosmet Toxicol 11:555–564 (1973).
84. Fabian RJ, Abraham R, Coulston F, Golberg L. Carrageenan-induced squamous metaplasia of the rectal mucosa in the rat. Gastroenterology 65:265–276 (1973).
85. Abraham R, Golberg L, Coulston F. Uptake and storage of degraded carrageenan in lysosomes of reticuloendothelial cells of the rhesus monkey. Exp Mol Pathol 17:77–93 (1972).
86. Watt J, Marcus R. Carrageenan-induced ulceration of the large intestine in the guinea pig. Gut 12:164–171 (1971).
87. Marcus R, Watt J. Colonic ulceration in young rats fed degraded carrageenan. Lancet 2:765–766 (1971).
88. Sharratt M, Grasso P, Carpanini F, Gangolli SD. Carrageenan ulceration as a model for human ulcerative colitis. Lancet 2:932 (1970).
89. Watt J, Marcus R. Ulcerative colitis in rabbits fed degraded carrageenan. J Pharm Pharmacol 22:130–131 (1970).
90. Watt J, Marcus R. Hyperplastic mucosal changes in the rabbit colon produced by degraded carrageenin. Gastroenterology 59:760–768 (1970).
91. Maillet M, Bonfils S, Lister RE. Carrageenan: effects in animals. Lancet 2:414–415 (1970).
92. Watt J, Marcus R. Ulcerative colitis in the guinea-pig caused by seaweed extract. J Pharm Pharmacol 21:187S–188S (1969).
93. Marcus R, Watt J. Seaweeds and ulcerative colitis in laboratory animals. Lancet 2:489–490 (1969).
94. Onderdonk AB. The carrageenan model for experimental ulcerative colitis. Prog Clin Biol Res 186:237–245 (1985).
95. Ottet NK. On animal models for inflammatory bowel disease. Gastroenterology 62:1269–1272 (1972).
96. Watt J, Marcus R. Progress report: Experimental ulcerative disease of the colon in animals. Gut 14:506–510 (1973).
97. Sharratt M, Grasso P, Carpanini F, Gangolli SD. Carrageenan ulceration as a model for human ulcerative colitis. Lancet 1:192–193 (1971).
98. Mottet NK. On animal models for inflammatory bowel disease. Gastroenterology 62:1269–1271 (1971).
99. Kim H-S, Berstad A. Experimental colitis in animal models. Scand J Gastroenterol 27:529–537 (1992).
100. Watt J, Marcus SN, Marcus AJ. The comparative prophylactic effects of sulfasalazine, prednisolone, and azathioprine in experimental ulceration. J Pharm Pharmacol 32:873–874 (1980).
101. Kitano A, Matsumoto T, Oshitani N, Nakagawa M, Yasuda K, Watanabe Y, Tomobuchi M, Obayashi M, Tabata A, Fukushima R, et al. Distribution and anti-inflammatory effect of mesalazine on carrageenan-induced colitis in the rabbit. Clin Exp Pharmacol Physiol 23:305–309 (1996).
102. Ishioka T, Kuwabara N, Oohashi Y, Wakabayashi K. Induction of colorectal tumors in rats by sulfated polysaccharides. CRC Crit Rev Toxicol 17:215–244 (1987).
103. Gangolli SD, Wright MG, Grasso P. Identification of carrageenan in mammalian tissues: an analytical and histochemical study. Histochem J 5:37–48 (1973).
104. Pipy B. 9-Carraghénanes et macrophages. Sciences des aliments 4:415–428 (1984).
105. Catanzaro PJ, Schwartz HJ, Graham RD. Spectrum and possible mechanism of carrageenan cytotoxicity. Am J Pathol 64:387–404 (1971).
106. Thomson AW, Fowler EF. Carrageenan: a review of its effect on the immune system. Agents Actions 1:265–273 (1981).
107. Kolodny EW, Fluharty AL. Metachromatic leukodystrophy and multiple sulfatase deficiency: sulfatide lipidosis. In: The Metabolic and Molecular Bases of Inherited Diseases (Scriver CR, AL Beaudet AL, Sly WS, Valle D, eds). 7th ed. New York: McGraw-Hill, Inc., 1995; 2693–2739.
108. Ballabio A, Shapiro LJ. Steroid sulfatase deficiency and X-linked ichthyosis. In: The Metabolic and Molecular Bases of Inherited Diseases (Scriver CR, Beaudet AL, Sly WS, Valle D, eds) 7th ed. New York: McGraw-Hill, Inc., 1995;2999–3022.
109. Cotran RS, Kumar V, Robbins SL, Schoen FJ. Genetic diseases. Robbins' Pathological Basis of Disease. 5th ed. Philadelphia:W.B. Saunders Company, 1994;123–171.
110. Muenzer J. Mucopolysaccharidoses. Adv Pediatr 33:269–302 (1986).
111. Corpet DE. Toxicological evaluation of carrageenans. 5-Dietary carrageenans and intestinal microflora. Sciences des aliments 4:367–374 (1984).
112. Michel C, Macfarlane GT. Digestive fates of soluble polysaccharides from marine macroalgae: involvement of the colonic microflora and physiological consequences for the host. J Appl Bacteriol 1996;80:349–369 (1996).
113. Gibson GR, Macfarlane S, Cummings JH. The fermentability of polysaccharides by mixed human faecal bacteria in relation to their suitability as bulk-forming laxatives. Lett Appl Microbiol 11:251–254 (1990).
114. Roediger WEW, Duncan A, Kapaniris O, Millard S. Reducing sulfur compounds of the colon impair colonocytes nutrition: implications for ulcerative colitis. Gastroenterology 104:802–809 (1993).
115. Richardson CJ, Magee EAM, Cummings JH. A new method for the determination of sulphide in gastrointestinal contents and whole blood by microdistillation and ion chromatography. Clin Chim Acta 293:115–125 (2000).
116. Babidge W, Millard S, Roediger W. Sulfides impair short chain fatty acid beta-oxidation at acyl-CoA dehydrogenase level in colonocytes: implications for ulcerative colitis. Mol Cell Biochem 181:117–124 (1998).
117. Toscani A, Soprano DR, Soprano KJ. Molecular analysis of sodium butyrate-induced growth arrest. Oncogene Res 3:223–238 (1998).
118. Glinghammar B, Holmberg K, Rafter J. Effects of colonic lumenal components on AP-1 dependent gene transcription in cultured human colon carcinoma cells. Carcinogenesis 20:969–976 (1999).
119. Salyers AA, West SHE, Vercelotti JR, Wilkins TD. Fermentation of mucins and plant polysacchairds by anerobic bacteria from the human colon. Appl Environ Microbiol 334:529–533 (1977).
120. Di Rosa M. Review: Biological properties of carrageenan. J Pharm Pharmacol 24:89–102 (1972).
121. Hoffman R. Carrageenans inhibit growth-factor binding. Biochem J 289:331–334 (1993).
122. Cochran FR, Baxter CS. Macrophage-mediated suppression of T-lymphocyte proliferation induced by oral carrageenan administration. Immunology 53:221–227 (1984).
123. Thomson AW, Fowler EF. Potentiation of tumor growth by carrageenan. Transplantation 24:397–400 (1977).
124. Carlucci MJ, Pujol CA, Ciancia M, Noseda MD, Matulewicz MC, Damonte EB, Cerezo AS. Antiherpetic and anticoagulant properties of carrageenans from the red seaweed *Gigartina skottsbergii* and their cyclized derivatives: correlation between structure and biological activity. Int J Biol Macromol 20:97–105 (1997).
125. Yamada T, Ogano A, Saito T, Watanabe J, Uchiyama H, Nakagawa Y. Preparation and anti-HIV activity of low-molecular-weight carrageenans and their sulfated derivatives. Carbohydr Polym 32:51–55 (1997).
126. Pearce-Pratt R, Phillips DM. Sulfated polysaccharides inhibit lymphocyte-to-epithelial transmission of human immunodeficiency virus-1. Biol Reprod 54:173–182 (1996).
127. Zaretzky FR, Pearce-Pratt R, Phillips DM. Sulfated polyanions block *Chlamydia trachomatis* infection of cervix-derived human epithelia. Infect Immun 63:3520–3526 (1995).
128. Hoffman R, Burns WW, Paper DH. Selective inhibition of cell proliferation and DNA synthesis by the polysulphated carbohydrate ι-carrageenan. Cancer Chemother Pharmacol 36:325–334 (1995).
129. Coombe DR, Parish CR, Ramshaw IA, Snowden JM. Analysis of the inhibition of tumour metastasis by sulphated polysaccharides. Int J Cancer 39:82–88 (1987).
130. Tobacman JK. Filament disassembly and loss of mammary myoepithelial cells after exposure to lambda-carrageenan. Cancer Res 57:2823–2826 (1997).
131. Tobacman JK, Walters K. Carrageenan exposure leads to mammary myoepithelial cell development of unusual intracellular inclusions. Proc Am Assoc Cancer Res 39:4722 (1999).
132. Cater DB. The carcinogenic action of carrageenin in rats. Br J Cancer 15:607–614 (1961).
133. Hopkins J. Carcinogenicity of carrageenan. Food Cosmet Toxicol 19:779–788 (1981).
134. Dyrset N, Lystad KQ, Levine DW. Development of a fermentation process for production of a kappa-carrageenase from *Pseudomonas carrageenovora*. Enzyme Microb Technol 20(6):418–423 (1997).
135. Irvine EJ, Farrokhyar F, Swarbrick ET. A critical review of epidemiological studies in inflammatory bowel disease. Scand J Gastroenterol 36(1):2–15 (2001).
136. Ferlay J, Bray F, Pisani P, Parkin DM. GLOBOCAN 2000: Cancer Incidence, Mortality and Prevalence Worldwide, Version 1.0. IARCCancerBase No. 5. Lyon:IARC Press, 2001. Limited version available: http://www-dep.iarc.fr/cgi-bin/exe-globom.exe [cited 2 March 2001].
137. Gold LS, Slone TH, Manley NB, Garfinkel GB, Rohrbach L, Ames BN. Carcinogenic potency database. In: Handbook of Carcinogenic Potency and Genotoxicity Databases (Gold LS, Zeiger E, eds). New York:CRC Press, Inc., 1997;116–117.
138. Gold LS, Slone TH, Ames BN. Summary of carcingogenic potency database by chemical. In: Handbook of Carcinogenic Potency and Genotoxicity Databases (Gold LS, Zeiger E, eds). New York:CRC Press, Inc., 1997;629.
139. Food Additives Amendment of 1958. Public Law 85–929, 72 Stat. 1784.
140. Food Quality Protection Act of 1996. Public Law 104–170, 110 Stat. 1489.

Review of Harmful Gastrointestinal Effects of Carrageenan in Animal Experiments

Joanne K. Tobacman

College of Medicine, University of Iowa, Iowa City, Iowa, USA

In this article I review the association between exposure to carrageenan and the occurrence of colonic ulcerations and gastrointestinal neoplasms in animal models. Although the International Agency for Research on Cancer in 1982 identified sufficient evidence for the carcinogenicity of degraded carrageenan in animals to regard it as posing a carcinogenic risk to humans, carrageenan is still used widely as a thickener, stabilizer, and texturizer in a variety of processed foods prevalent in the Western diet. I reviewed experimental data pertaining to carrageenan's effects with particular attention to the occurrence of ulcerations and neoplasms in association with exposure to carrageenan. In addition, I reviewed from established sources mechanisms for production of degraded carrageenan from undegraded or native carrageenan and data with regard to carrageenan intake. Review of these data demonstrated that exposure to undegraded as well as to degraded carrageenan was associated with the occurrence of intestinal ulcerations and neoplasms. This association may be attributed to contamination of undegraded carrageenan by components of low molecular weight, spontaneous metabolism of undegraded carrageenan by acid hydrolysis under conditions of normal digestion, or the interactions with intestinal bacteria. Although in 1972, the U.S. Food and Drug Administration considered restricting dietary carrageenan to an average molecular weight > 100,000, this resolution did not prevail, and no subsequent regulation has restricted use. Because of the acknowledged carcinogenic properties of degraded carrageenan in animal models and the cancer-promoting effects of undegraded carrageenan in experimental models, the widespread use of carrageenan in the Western diet should be reconsidered. *Key words*: carcinogenesis, carrageenan, carrageenase, diet, furcelleran (furcellaran), hydrolysis, inflammatory bowel disease, nutrition, poligeenan, promoter, sulfated polysaccharide. *Environ Health Perspect* 109:983–994 (2001). [Online 24 September 2001]
http://ehpnet1.niehs.nih.gov/docs/2001/109p983-994tobacman/abstract.html

During the latter half of the twentieth century, inflammatory bowel disease and gastrointestinal malignancy have been major causes of morbidity and mortality in the United States. Even with improvements in treatment and cancer screening, colorectal cancer remains the second leading cause of cancer mortality in the United States. The Western diet has been considered a possible source of inflammatory bowel disease and colorectal malignancy, and intensive efforts have been undertaken to study the impact of specific constituents of the Western diet, such as fiber and fat (*1–3*).

One food additive, carrageenan, has been associated with induction and promotion of intestinal neoplasms and ulcerations in numerous animal experiments; however, carrageenan remains a widely used food additive. In 1982, the International Agency for Research on Cancer (IARC) (*4*) designated degraded carrageenan as Group 2B, noting sufficient evidence for the carcinogenicity of degraded carrageenan in animal models to infer that "in the absence of adequate data on humans, it is reasonable, for practical purposes, to regard chemicals for which there is sufficient evidence of carcinogenicity in animals as if they presented a carcinogenic risk to humans" (p. 90). The National Research Council has noted this designation for degraded carrageenan in their 1996 monograph (*5*). Recognizing the impact of carrageenan in animal models, several European and British investigators have advised against the continued use of carrageenan in food (*6–11*). Several reports have called attention to the problems associated with carrageenan consumption (*6–11*).

Extracted from red seaweed, carrageenan has been used in food products for centuries and was patented as a food additive for use in the United States in the 1930s. It has been used widely as a food additive, contributing to the texture of a variety of processed foods. It has also been used as a laxative, as treatment for peptic ulcer disease, and as a component of pharmaceuticals, toothpaste, aerosol sprays, and other products (*12–15*). In 1959, carrageenan was granted GRAS (Generally Regarded as Safe) status in the United States. GRAS substances are permitted to be incorporated into food products as long as good manufacturing processes are used and the substance is used only in sufficient quantity to achieve the desired effect (*16,17*).

In the United States, the status of carrageenan was reconsidered by the Food and Drug Administration, and an amendment to the Code of Federal Regulations for the food additive carrageenan was proposed in 1972 (*18*). To diminish the public's exposure to degraded carrageenan, the amendment supported inclusion of an average molecular weight for carrageenan of 100,000 and a minimum viscosity of 5 centipoises (cps) under specified conditions. However, the actual regulation was not amended, although several publications indicated that it had been modified (*7,8,19–23*). In 1979, the proposal to include the average molecular weight requirement of 100,000 and the associated viscosity requirement in the Code of Federal Regulations was withdrawn. It was anticipated that a new rule-making proposal on carrageenan that would comprehensively address all food safety aspects of carrageenan and its salts would be published in about a year, but this has not been forthcoming (*24,25*). The proposal withdrawal referred to interim specifications for food-grade carrageenan using the Food Chemical Codex; these include a viscosity stipulation, but no average molecular weight requirement (*26*).

In the Food Chemicals Codex and supplements, carrageenan is described with attention to specific requirements for its identification and tests of its properties, including its sulfate content, heavy metal content, solubility in water, content of acid-insoluble matter, and viscosity [a 1.5% solution is to have viscosity ≥ 5 cps at 75°C] (*26,27*). Although the viscosity is stipulated, viscosity may not adequately protect food-grade carrageenan from contamination by the lower molecular weight degraded carrageenans that IARC has denoted as Group 2B. Because undegraded carrageenan may have molecular weight in the millions, the actual viscosities of commercial carrageenans range from about 5 to 800 cps when measured at 1.5% at 75°C (*14*). Native carrageenan has molecular weights of 1.5×10^6–2×10^7 (*28*); poligeenan or degraded carrageenan is described as having average molecular weight of 20,000–30,000 (*4*). The average molecular weight of poligeenan has been described elsewhere as 10,000–20,000, but extending up to 80,000 (*29*). Food-grade carrageenan has been

Address correspondence to J.K. Tobacman, Department of Internal Medicine, University of Iowa Health Care, 200 Hawkins Drive, Iowa City, Iowa 52242-1081, USA. Telephone: (319) 356-3702. Fax: (319) 356-3086. E-mail: joanne-tobacman@uiowa.edu

Received 17 January 2001; accepted 17 March 2001.

described as having average molecular weight of 200,000–400,000 (*29*), and elsewhere as having molecular weight of 100,000–800,000 (*19*). Furcelleran (or furcellaran), a degraded carrageenan of molecular weight 20,000–80,000, has a sulfate content of 8–19% (*12,17*). No viscosity or minimum average molecular weight was designated for furcelleran in the 1972 or 1979 Federal Register documents (*18,24*). In the Food Chemical Codex (fourth edition), a 1.5% solution of furcelleran at 75°C is described as having minimum viscosity of 5 cps (*27*).

Today, carrageenan is still included among the food additives designated GRAS in the Code of Federal Regulations. The stipulations for its use include the following: *a*) it is a sulfated polysaccharide, the dominant hexose units of which are galactose and anhydrogalactose; *b*) range of sulfate content is 20–40% on a dry-weight basis; *c*) the food additive is used or intended for use in the amount necessary for an emulsifier, stabilizer, or thickener in foods, except for those standardized foods that do not provide for such use; *d*) to assure safe use of the additive, the label and labeling of the additive shall bear the name of the additive, carrageenan. Also included are similar standards for carrageenan salts and for furcelleran and furcelleran salts (*30*). In 1999–2000, approved uses for carrageenan were extended to include additional incorporation into food and medicinal products, including both degraded and undegraded carrageenan in laxatives (*31–33*).

For use in experimental models, degraded carrageenan (poligeenan) is derived from carrageenan by acid hydrolysis, frequently by a method developed by Watt et al. (*34*). This method is expected to yield a degraded carrageenan of average molecular weight 20,000–30,000 (*35*). Experiments demonstrate that reaction conditions similar to those of normal digestion can lead to the formation of degraded carrageenan (*9–11*). In addition, experimental data have revealed the contamination of food-grade carrageenan by substantial amounts of degraded carrageenan (*10*). Also, some bacteria are known to hydrolyze carrageenan and form low molecular weight derivatives (*36–40*).

The sections that follow and the accompanying tables summarize many experimental observations with regard to the intestinal effects of carrageenan. In addition, I review possible mechanisms for production of degraded carrageenan from undegraded carrageenan under physiologic conditions, as well as evidence that provides a basis for the mechanism of carrageenan's effects and for the reconsideration of the safety of carrageenan in the human diet.

Characteristics of Carrageenan

Three forms of carrageenan predominate, known as kappa, iota, and lambda. All have similar D-galactose backbones (alternating α-1,3 to β-1,4 linkages), but they differ in degree of sulfation, extent of branching, solubility, cation binding, and ability to form gels under different conditions. λ-Carrageenan is the least branched and the least gel forming; it is readily soluble at cold temperatures, in contrast to κ- or ι-carrageenan. Table 1 presents some of the basic characteristics of κ, ι, and λ carrageenan (*4,12–15,20–22,31–33,41–44*)

In addition to food additive uses, carrageenan has been used in cosmetics, pesticides, and pharmaceuticals, as well as in toothpaste and room deodorizers. It has been used as a treatment of ulcers and as an emulsifier in mineral oil laxatives, liquid petrolatum, and cod liver oil. However, its predominant role has been in food preparations, in which it is used across a wide variety of food groups because of its ability to substitute for fat and its ability to combine easily with milk proteins to increase solubility and improve texture. Hence, it is used in low-calorie formulations of dietetic beverages, infant formula, processed low-fat meats, whipped cream, cottage cheese, ice cream, and yogurt, as well as in other products. From its original use several centuries ago as a thickener in Irish pudding and its incorporation into blancmange, the food additive use has extended widely and cuts across both low-fat and high-fat diets. It is often combined with other gums, such as locust bean gum, to improve the texture of foods (*12–14,22,41,42*).

In 1977, data obtained by the survey of industry on the use of food additives produced an estimate of daily carrageenan intake of 100 mg for individuals older than 2 years. The 1971 survey of industry had indicated that formula-fed infants in the first 5 months of life had an intake of 108 mg/day (*21,43*). Informatics, Inc., in a report prepared for the Food and Drug Administration, cited daily carrageenan consumption of 45 mg (*19*); this is similar to the reported intake of 50 mg/day of carrageenan in France (*45*). Nicklin and Miller (*20*) reported intake of 0–1.5 g/day, depending on choice of diet and total food consumed. Although the Food and Nutrition Board of the National Research Council of the National Academy of Sciences of the United States in 1971 initially estimated 367 mg/day for carrageenan intake for individuals older than 2 years in the United States, this was subsequently revised to 11 mg/day. The wide range of estimates may be attributed to inconsistencies in how industry has reported carrageenan production and consumption data, variation in processed food formulations with regard to extent of incorporation of carrageenan, and changes in use of carrageenan in nonfood products. Daily individual consumption of between 50 mg/day and 100 mg/day is consistent with total consumption in the United States of 7,700 metric tons, as estimated for 1997 (*46*).

The content of carrageenan in several commonly consumed food products is summarized

Table 1. Characteristics of carrageenan (*4,12–15,27,28,41–49*).

Chemical composition	Hydrocolloid composed of α-D-1,3 and β-D-1,4 galactose residues that are sulfated at up to 40% of the total weight. Strong negative charge over normal pH range. Associated with ammonium, calcium, magnesium, potassium, or sodium salts.
Solubility	λ is readily soluble in cold or hot aqueous solution; κ is soluble in hot solution; treatment of aqueous solution with potassium ion precipitates κ-carrageenan.
Gel formation	λ does not form gels; λ and ι form right-handed helices; potassium chloride promotes gel formation of κ; calcium ion promotes gel formation of ι.
Metabolism	Hydrolysis of glycosidic linkages at lower pH, especially pH ≤ 3.0; also desulfation by sulfatases.
Viscosity	Near logarithmic increase in viscosity with increasing concentration. Viscosity of food-grade carrageenan defined as not less than 5 cps at 75°C for a 1.5% solution; viscosity ranges from 5 to 800 cps for 1.5% solution at 75°C.
Source	Red algae; predominantly aqueous extraction from *Chondrus, Gigartina,* and various *Eucheuma* species.
Molecular weight	Discrepancies in definitions. Native carrageenan reported to have average molecular weight of $1.5 \times 10^6 - 2 \times 10^7$; food-grade carrageenan reported as 100,000–800,000 or 200,000–400,000. Degraded carrageenan (poligeenan) has average molecular weight of 20,000–30,000; furcellaran has average molecular weight 20,000–80,000.
Properties	λ and κ combine easily with milk proteins to improve solubility and texture; serve as thickening agent, emulsifier, stabilizer.
Synergistic effects	With locust bean gum, increase in gel strength. Other hydrocolloids may also affect gel strength and cohesiveness.
Concentration in food products	0.005–2.0% by weight.
Major uses	Milk products, processed meats, dietetic formulations, infant formula, toothpaste, cosmetics, skin preparations, pesticides, laxatives

in Table 2. Because manufacturing practices vary and change over time and the food formulae are proprietary, carrageenan content is indicated by a range (*12,13,47–49*). The content is expressed as the percent by weight of carrageenan used in the production of the food.

Experimental Results in Animal Models

Intestinal lesions after exposure to carrageenan in animal models. Table 3 summarizes the laboratory investigations that associate exposure to carrageenan with the occurrence of intestinal lesions (*50–93*). Several animals were tested, including guinea pig, rat, monkey, mouse, rabbit, and ferret. The guinea pig seemed most susceptible to ulceration and the rat most susceptible to malignancy. Many studies used exposure to carrageenan in a drinking fluid, at concentrations generally of 1%. Some were feeding studies, in which carrageenan was added to a solid diet. Some studies used gastric or duodenal intubation to ensure intake at a specified level; however, this method may have affected the way that carrageenan was metabolized by gastric acid (*74,82–84,91*). Feeding of carrageenan with milk may also have affected study results, because carrageenan binds tightly to milk proteins (caseins), affecting its metabolism (*12–15, 22,41,42,47*). The degraded carrageenan used in most of the experiments had molecular weight from 20,000 to 40,000. Several major findings in relation to neoplasia and ulceration were observed in these animal studies. All of these studies observed the effects of carrageenan in comparison to appropriate control animals.

In the footnote to Table 3, several subdivisions of the table are indicated with citation of the entries from the table. The subdivisions include: *a*) studies in which carrageenan alone induces abnormal proliferation or malignancy, *b*) studies in which carrageenan alone induces intestinal ulcerations, *c*) studies in which carrageenan appears to be a promoter of malignancy in association with another agent, *d*) studies using a rat model, *e*) studies using a guinea pig model, *f*) studies using degraded carrageenan, *g*) studies using undegraded carrageenan, *h*) studies indicating uptake of carrageenan into an extraintestinal site(s), *i*) studies indicating intestinal breakdown of carrageenan into lower molecular weight forms, and *j*) studies demonstrating ulcerations in rats using degraded carrageenan. In the table, the classification of the carrageenan used in the experiments as κ, λ, or ι is indicated when this information is clear from the original report.

Neoplasia. Wakabayashi and associates (*72*) demonstrated the appearance of colonic tumors in 32% of rats fed 10% degraded carrageenan in the diet for less than 24 months. The lesions included squamous cell carcinomas, adenocarcinomas, and adenomas. With exposure to 5% degraded carrageenan in drinking water, there was a 100% incidence of colonic metaplasia after 15 months. Metastatic squamous cell carcinoma was observed in retroperitoneal lymph nodes in this experiment. In addition, macrophages that had metachromatic staining consistent with carrageenan uptake were observed in liver and spleen.

Other studies have demonstrated the development of polypoidal lesions and marked, irreversible squamous metaplasia of the rectal mucosa, the extent of which was associated with duration and concentration of carrageenan exposure (*67,70*). Oohashi et al. (*67*) observed a 100% incidence of colorectal squamous metaplasia that progressed even after degraded carrageenan intake was discontinued in rats fed 10% degraded carrageenan for 2, 6, or 9 months and sacrificed at 18 months.

Fabian et al. (*84*) observed adenomatous and hyperplastic polyps as well as squamous metaplasia of the anorectal region and the distal colon in rats given 5% carrageenan as a drinking fluid. Similarly, Watt and Marcus (*90*) observed hyperplastic mucosal changes and polypoidal lesions in rabbits given carrageenan as drinking fluid for 6–12 weeks at a concentration of 0.1–5%. Focal and severe dysplasia of the mucosal epithelium was observed in rabbits after 28 months of 1% degraded carrageenan in their drinking fluid (*58*).

Promotion of neoplasia. Several studies demonstrated an increased occurrence of neoplasia in relation to exposure to undegraded or degraded carrageenan and associated exposure to a known carcinogen. Experimental data with undegraded carrageenan included enhanced incidence of colonic tumors in rats treated with azoxymethane (AOM) and nitrosomethylurea (NMU), when carrageenan was added to the diet. Groups of rats received control diet; control diet with 15% carrageenan; 15% carrageenan plus 10 injections of AOM given weekly; carrageenan plus NMU; NMU alone; and AOM alone. AOM or NMU with carrageenan led to 100% incidence of tumors, versus 57% with AOM alone and 69% with NMU alone ($p < 0.01$). Controls had 0%, and carrageenan alone led to an incidence of 7%. In addition, when undegraded carrageenan was combined with AOM, there was a 10-fold increase in the number of tumors per rat (*73*). (Figure 1)

Using undegraded carrageenan as a solid gel at concentration 2.5% for 100 days, Corpet et al. (*50*) found that after exposure to azoxymethane, there was promotion of aberrant crypt foci by 15% ($p = 0.019$). Exposure of rats to 6% undegraded carrageenan in the diet for 24 weeks, with 1,2-dimethylhydrazine (1,2-DMH) injections weekly, was associated with an increase in tumors from 40% to 75% and with the more frequent occurrence of larger and proximal tumors (*57*).

Degraded carrageenan in the diet of rats at a 10% concentration in association with exposure to 1,2-DMH weekly for 15 weeks was associated with an increase in small intestinal tumors from 20% to 50% and in colonic tumors from 45% to 60% (*64*). Iatropoulos et al. (*77*) found that in rats given 5% degraded carrageenan in the drinking water for less than 30 weeks in association with injections of 1,2-DMH weekly, there were increases in poorly differentiated adenocarcinomas and in tumors of the ascending and transverse colon, as well as increased proliferation of cells in the deep glandular areas.

Several investigators have measured the effect of carrageenan on thymidine incorporation and colonic cell proliferation. Wilcox et al. (*51*) observed a 5-fold increase in

Table 2. Range of content of carrageenan in some commonly consumed foods.

Food	Percent carrageenan (g/100 g food)
Bakery products	0.01–0.1
Chocolate milk	0.01–0.2
Cottage cheese	0.02–0.05
Frosting base mix	3–4
Ice cream, frozen custard, sherbets, etc.	0.01–0.05
Jams and jellies	0.5–1.2
Liquid coffee whitener	0.3
Pie filling	0.1–1.0
Pimento olive stuffing	2.0
Processed cheese	0.01–0.06
Processed meat or fish	0.2–1.0
Pudding (nondairy)	0.1–0.5
Relishes, pizza, barbecue sauces	0.2–0.5
Yogurt	0.2–0.5

Because manufacturing processes vary and there can be substitutions of one hydrocolloid for another, the content of carrageenan for any individual product may differ from these estimates. Unpublished manufacturers' data indicate that these content estimates for processed cheese, frozen dairy dessert, cottage cheese, and jams and jellies are significantly lower than current usage (*4,13,14,47*).

thymidine kinase activity in colon cells with 5% undegraded or 5% degraded carrageenan. There was an associated 35-fold increase in proliferating cells in the upper third of crypts with degraded carrageenan and an 8-fold increase with undegraded carrageenan (*51*). With 5% λ-undegraded carrageenan fed to rats for 4 weeks, Calvert and Reicks (*55*) observed a 4-fold increase in thymidine kinase activity in the distal 12 cm of the colon ($p < 0.001$). Fath et al. (*59*) observed a 2-fold increase in colonic epithelial cell proliferation,

Table 3. Experimental data related to intestinal effects of dietary carrageenan exposure.

Experiment							Effects			
Type of carrageenan, molecular weight	Animal	Dose %CG	Dose (g/kg bw/day)	Duration	Route	Additional exposure	Digestive/ systemic	Histopathologic changes	Neoplastic changes	Reference
1. Undegraded[a,d]	Rat	10	27.4	8 days, sacrificed at 30 days	Jelly	AOM injection (5 mg/kg ip)	Less weight gain with 2.5% CG		UCG jelly (10% × 8 days) did not initiate tumor.	(*50*)
Undegraded[a,d]	Rat	0.25 2.5	0.2 4	100 days 100 days	Liquid Solid gel	AOM injection (20 mg/kg ip) prior to CG			UCG solid gel promoted growth of aberrant crypt foci (+15%; $p = 0.019$).	
2. Undegraded ι, >100,000[a,b]	Rat	0.5, 1.5, 5		≤ 91 days	Diet			Epithelial cell loss, macrophage infiltration, loss of crypts.	5-Fold increase in thymidine kinase activity in colon cells with 5% UCG or DCG. 35-Fold increase in proliferating cells in upper third of crypt with DCG, 8-fold with UCG.	(*51*)
Degraded ι, 20,000[a,b]	Rat	0.5, 1.5, 5		≤ 91 days	Diet					
3. Degraded ι, 30,000[c]	Guinea pig	2, 2.5, 5		7 days	Drink	Eicosapentaenoic acid (300 mg/kg/day) at 14 days	2% led to FOB+ at 7 days; 5% led to 38% mortality	Cecal ulcerations; foamy macrophages; small epithelial ulcerations at 2 days.		(*52*)
4. Degraded, 20,000–30,000[c]	Guinea pig	3	5.8	2–3 days	Drink			Microscopic mucosal changes from cecum to rectum; apparent macromolecule absorption by colonic epithelium, macrophage infiltration, macrophages with vacuoles.		(*35*)
5. Degraded[c]	Guinea pig	1, 2, 3	2, 3, 4	2 weeks	Drink			100% had cecal ulceration after 3% for 4 days; crypt abscesses.		(*53*)
6. Degraded[c]	Rat ileal cell monolayers		0–1.5 g/L	19, 30, 54 hr	Media				Retarded cell growth caused cell death; at 0.25g/L inhibited DNA synthesis by 20%.	(*54*)
7. Undegraded λ, 300,000[a,b]	Rat	5		4 weeks	Diet			3/8 had slight congestion and erythema of distal colon.	4-Fold increase in thymidine kinase activity in distal 12 cm of colonic mucosa.	(*55*)
8. Degraded ι[c]	Rat, Guinea pig	5 5		4 months 3 weeks	Drink Drink			Increased permeability to (^{3}H) PEG-900; ulcerations in guinea pig, crypt abscesses, macrophage infiltration.		(*56*)
9. Undegraded κ[a,d]	Rat	6	0.8	24 weeks	diet	1,2-DMH (20 mg/kg bw) SC × 16 wks			More tumors with UCG than control diet (75% vs. 40%); also larger, more proximal tumors.	(*57*)
10. Degraded λ[a,b,c]	Rabbit	1		8 weeks, 12 months, 28 months	Drink			Ulcerative lesions at 8 wks; at 12 months had chronic inflammatory changes.	At 28 months, focal and severe glandular atypism; precancerous changes seen.	(*58*)
11. Degraded[a,b,c]	Mice	10		10 days	Drink		Bloody diarrhea	Ulceration in proximal and distal colon, with dilatation of cecum and ascending colon.	2-Fold increase in colonic epithelial cell proliferation; increase in labeling indices and extension of proliferative compartment to upper third of crypt.	(*59*)
12. Degraded, (20,000–40,000), and Undegraded[a]	Cultured rat hepatocytes or intestinal mucosal cells		1 mg/10 mL or 1 mg/100 mL	20 hr 2 hr					DCG and UCG nonmutagenic in *Salmonella* mutagenicity test; DCG nongenotoxic by DNA repair test.	(*60*)
13. Undegraded κ, λ, and ι[e]	Rat	0.5	0.15–0.25	90 days	Drink			CG able to penetrate intestine; CG in mesenteric lymph node, and macrophages of villus and lamina propria.		(*61*)
14. Degraded[c]	Guinea pig	5		5 days	Drink			Small, superficial ulcerations over mucosal surface of cecum (1–111 ulcerations/cm^2).		(*62*)
15. Degraded[c]	Guinea pig	5		14 days	Drink	With ileotransversostomy		Ulcerations in cecum and proximal colon in unoperated. Postprocedure, crypt abscesses in rectum and ulcerations in distal colon and rectum. Macrophage infiltration.		(*63*)

Continued, next page

with increase in labeling indices in both proximal and distal colon and extensive increase of the proliferative compartment in the proximal colon to the upper third of the intestinal crypt, after exposure of mice to 10% degraded carrageenan in drinking water for 10 days.

Ulceration. Many studies have demonstrated significant ulceration of the cecum and/or large intestine after oral exposure to carrageenan in guinea pigs, rabbits, mice, rats, and rhesus monkeys (*34,35,53,56,58,59,62,63,65,68,70,71,75,*

Table 3. *Continued*

Experiment							Effects			
Type of carrageenan, molecular weight	Animal	%CG	Dose (g/kg bw/day)	Duration	Route	Additional exposure	Digestive/ systemic	Histopathologic changes	Neoplastic changes	Reference
16. Degraded[a,d]	Rat	10		2 weeks	Diet	1,2-DMH weekly injections for 15 weeks (10 mg/kg bw) with DCG vs. DMH alone			Increase in tumors of small intestine (50% vs. 25%) and colon (60% vs. 45%) with CG than occurred with DMH alone.	(*64*)
17. Degraded, (20,000–40,000)[a,b,c,g]	Rat	10	15	< 63 days	Diet	Germ-free vs. conventional gut flora		Erosions; aggregates of foamy metachromatic macrophages in submucosa and lamina propria.	Squamous metaplasia from anorectal junction to distal colon.	(*65*)
18. Degraded κ, λ, ι	Guinea pig	3		3 weeks	Drink		Diarrhea after 7 days. Moribund after 9 days of ιDCG.	No cecal or colonic lesions seen.		(*66*)
19. Degraded (20,000–40,000)[a,b,e]	Rat	10		2, 6, 9 months sacrificed at 18 months	Diet	Basal diet after DCG exposure		CG in mucosa and RE system.	100% incidence of colorectal squamous metaplasia that progressed after DCG intake discontinued.	(*67*)
20. Degraded[c]	Guinea pig	5		≤ 28 days	Drink			Cecal lesions after 24 hr; confluent ulcerations after 7 days. Macrophage infiltration.		(*68*)
21. Undegraded, (800,000) (largely κ)	Rat, hamster	0.5, 2.5, 5	0.36, 2, 4	Lifetime	Diet		Diarrhea in some	No difference in ulcerations from control.	Increased incidence of benign mammary tumors and testicular neoplasms (at 2.5% level) in rats only.	(*69*)
22. Degraded[a,b,c,g]	Rat	10		1 day to 12 weeks, some sacrificed at 27 weeks	Diet			Superficial erosions at anorectal junction at 24 hr; at 2 weeks, more proximal erosions.	Squamous metaplasia of rectal mucosa at 2 weeks; extended after no longer being fed CG.	(*70*)
23. Degraded[c]	Guinea pig	2		2 weeks	Drink		Loose stools by 2 weeks	100% with colonic ulcerations; 75% had over 200 ulcers.		(*34*)
24. Degraded[c]	Guinea pig	5		21 days			17/22 died by day 21	All had mucosal ulcerations from cecum to rectum by day 14.		(*71*)
25. Degraded (20,000–40,000)[a,b]	Rat	10, 5, 1 5 1 or 5		≤ 24 months 15 months 15 months	Diet drink Stomach tube				Squamous cell carcinomas, adenocarcinomas, adenomas. 32% and fed 10% diet had tumors. 100% incidence of metaplasia with 5% drink.	(*72*)
26.Undegraced λ[a,d]	Rat	15		≤ 40 weeks	Diet	NMU 2 mg twice weekly rectally for 3 weeks; AOM (8 mg/kg bw) SC for 10 weeks; UCG with NMU, UCG with AOM			100% had tumors with AOM and UCG vs. 57% with AOM alone. 100% had tumors with NMU and UCG vs. 69% with NMU alone. 0 tumors in control, 7% tumors with UCG alone. UCG with AOM had 10-fold increase in number of tumors per rat.	(*73*)
27. ι, (8,700–145,000)[e,f]	Rat		0.5	9 months	Gavage			Av MW of CG in liver was at 10,000; all CG in feces had MW < 100,000.		(*74*)
Undegraded κ/λ, (186,000–214,000)	Rat	5		13 weeks	Diet					
ι, (5,000–145,000)	Guinea pig	2		7–10 weeks	Diet					
ι, (5,000–145,000)	Guinea pig	1		2–3 weeks	Drink					
κ, 8,500–275,000	Guinea pig	1		2–3 weeks	Drink					
λ, (8,500–275,000)	Guinea pig	1		2–3 weeks	Drink					
Undegradec κ/λ, (185,000)	Rhesus monkey		0.05, 0.2, 0.5		Stomach tube					
ι			0.2		Stomach tube					

Continued, next page

78–80,82,83,86–93). Ulcerations arose in association with exposure to either degraded or undegraded carrageenan. Lesions occurred initially in the cecum of guinea pigs and rabbits, but could be induced in more distal parts of the colon of the guinea pig, as in an experiment in which carrageenan was introduced directly into the colon after ileotransversostomy (*63*). In rats, the ulcerative lesions appeared initially in distal colon and rectum (*8*). Undegraded and degraded carrageenan have been associated

Table 3. *Continued*

Experiment							Effects			
Type of carrageenan, molecular weight	Animal	%CG	Dose (g/kg bw/day)	Duration	Route	Additional exposure	Digestive/ systemic	Histopathologic changes	Neoplastic changes	Reference
28.κ[c] (314,000) (51,500) (8,500) λ, (275,000) (74,800) (20,800) ι, (145,000) (107,000) (88,000) (39,000) (21,000) (8,700) (5,000)	Guinea pig	1		2 weeks 2 weeks 2 weeks 2 weeks 2 weeks 2 weeks 2 weeks 10 weeks 2 weeks 10 weeks 2 weeks 2 weeks 10 weeks 2 weeks 10 weeks 2 weeks	Drink Drink Drink Drink Drink Drink Drink Diet Drink Diet Drink Drink Diet Drink Diet Drink			Cecal ulceration not seen with κ or λ; ι fractions of MW 21,000–107,000 led to ulcerations of cecum, crypt abscesses, and epithelial thinning. ι fractions absorbed and seen in vacuolated macrophages. Intense lysosomal enzymatic activity in macrophages of lamina propria.		(*75*)
29. Degraded ι (C16), (20,000)	Rhesus monkey	2		10 weeks	Drink			Macrophages given DCG had fibrillar material and vacuolations.		(*76*)
Undegraded κλ mixture, (800,000)	Rhesus monkey	1		10 weeks	Drink			Vacuolations seen with UCG.		
30. Degraded (ι, C16), (10,000–30,000)[a,d]	Rat	5	7.5 g, then 5 g	≤ 30 weeks	Drink, diet	1,2-DMH (20 mg/kg) SC/wk	Watery, bloody stools	Distal rectum transformed to stratified squamous by DMH with DCG.	DMH with DCG-induced proliferation of deep glandular areas; more poorly differentiated adenocarcinomas; more frequently found tumors of ascending and transverse colon with DMH and DCG.	(*77*)
31. Degraded[c,e]	Rat	0.2, 0.5, 5		≤ 12 weeks	Drink		Severe diarrhea in 3 days with 5%	DCG contained within macrophages of spleen, liver, kidney, small and large intestine; cecal and colonic ulcerations at 4 weeks.		(*78*)
	Guinea pig	0.25, 0.5		≤ 4 weeks	Drink		Diarrhea			
32. Degraded (ι, C16)[c]	Guinea pig	2, 0.2, 0.02		12 months, 10 months	Drink			2% CG in water, but not in milk, led to cecal ulceration in guinea pig. DCG in macrophages of submucosal layer in guinea pigs, rats, and monkeys. No cecal ulceration seen in rats or monkeys.		(*79*)
	Guinea pig	2		3 months	In milk					
	Rat	5		3 months	Drink					
	Monkey	2		10 weeks	Drink					
33. Degraded[c]	Guinea pig	2, 5	1.7–3.3	30–44 days	Drink	Trimethoprim/ Sulfamethoxazole	Blood in stools	Cecum and distal colon had ulcerations, crypt abscesses; enlarged cecal or colonic lymph nodes; more extensive ulceration with 5%; fewer lesions with antibiotic. Infiltration of foamy macrophages.		(*80*)
34. Undegraded κ, (200,000)	Pig		0.05, 0.2, 0.5	83 days	Jelly			Focal irregularities without ulcerations; thickened lamina propria; macrophage infiltration.		(*81*)
35. Degraded (C16, ι),[c] (20,000)	Rhesus monkey	0.5–2	0.7, 1.4, 2.9	7–14 weeks, then recovery for 20–24 weeks for some before sacrificed	Drink		Diarrhea, hemorrhage	Ulcerations of colon; hypertrophy of mesenteric lymph nodes and granulomas; multiple crypt abscesses; dose effect present.		(*82*)
Undegraded (largely κ), (800,000)	Rhesus monkey	1	1.3	7–14 weeks, then 11 weeks recovery	Drink			Without colonic changes.		
		1–3	0.05–1.25	≤ 12 weeks, after recovery	Tube					

Continued next page

with epithelial cell loss and erosions in rats (*51,65,70,87,93*).

Watt et al. (*34*) first observed ulcerations in response to carrageenan exposure in animal models more than three decades ago. They noted that 100% of guinea pigs given 2% degraded carrageenan as liquid for 20–30 days had colonic ulcerations and that 75% of the animals > 200 ulcers (*34*). When guinea pigs were given 1% undegraded carrageenan as liquid for 20–30 days, 80% developed colonic ulcerations (*92*). The

Table 3. *Continued*

Experiment							Effects			
Type of carrageenan, molecular weight	Animal	%CG	Dose (g/kg bw/day)	Duration	Route	Additional exposure	Digestive/ systemic	Histopathologic changes	Neoplastic changes	Reference
36. Undegraded	Guinea pig	5		1–45 days	Diet	Neomycin (0.1%) added	Diarrhea, hemorrhage	Multiple pinpoint cecal and colonic ulcerations after 3–5 weeks in guinea pig and rabbit. Macrophages increased; inclusions and vacuoles in macrophages; granulomas seen. Neomycin did not affect incidence of ulcers or time of onset.		(*83*)
	Rabbit	5			Diet					
Degraded[c]	Guinea pig	1, 2, 5		1–45 days	Drink					
	Rabbit	2			Drink					
Degraded	Humans		5-g dose	10 days	Diet			Patients had colon malignancy with colectomy planned to follow CG exposure, no ulcerations seen.		
	Ferret		1.5	28 days	Tube			No lesions seen.		
	Squirrel monkey		1.5	28 days	Tube			No lesions seen.		
	Rabbit, mouse		1.5	28 days	Tube			No lesions seen.		
	Rat	1		56 days	Drink		SI diarrhea			
	Rat	5					Diarrhea			
Undegraded	Rat	5		56 days	Diet		SI diarrhea			
	Hamster	5		6 months	Diet		Diarrhea			
37. Degraded (c16, ι), (20,000–30,000)[a,b,e]	Rat	5	6–10 0.5–5.0	≥ 25 weeks 1–15 months	Drink Tube		FOB+ by 3–7 days with > 5 g/kg/day; gross blood by 2–3 weeks	Metachromatic material thought to be CG found in RE cells of liver, spleen, lymph nodes, macrophages of lamina propria and submucosa. No cecal lesions.	Adenomatous and hyperplastic polyps in one rat. Squamous metaplasia of anorectal region and distal colon.	(*84*)
38. Undegraded (κ:λ = 70:30), (800,000)[e]	Rhesus monkey	1		7–12 weeks	Drink			No changes in liver.		(*85*)
Degraded (C16, ι), (20,000–30,000)[e]	Rhesus monkey	0.5, 1, 2		7–12 weeks	Drink			Membrane-bound vacuoles with fibrillar material in RE cells of liver.		
39. Degraded[c]	Guinea pig	5	≤ 2 g	20–45 days	Drink		FOB+, diarrhea by 1 week	Multiple ulcers in cecum, colon, and rectum in 100% of animals by day 30.		(*86*)
40. Degraded[a,b,c,g]	Rat	5		6 months	Drink			Ulceration of cecum in 4/12, associated with stricture; marked glandular hyperplasia at ulcer margins.		(*87*)
41. Undegraded[c]	Guinea pig	5		2–4 weeks	Diet			Ulceration of mucosa as consequence of macrophage accumulation in lamina propria, then submucosa.		(*88*)
Degraded[c]	Guinea pig	1		2–4 weeks	Drink					
42. Degraded[a,b,c]	Rabbit	0.1, 1, 5	0.07, 0.8, 1.4	6–12 weeks	Drink		Diarrhea, blood by day 7, weight loss	Ulceration of colon in 100% of those fed 1%; 60% of those fed 0.1%.	Hyperplastic mucosal changes, polypoidal lesions.	(*89,90*)
43. Degraded[c]	Guinea pig		4–5		Drink			Mucosal erosions in cecum, rarely into colon in guinea pig; without erosion in rat or mouse.		(*91*)
Degraded	Rat		≤ 16.5		Drink					
Degraded, Undegraded	Rat, mouse		0.07–4	28 days–6 months	Tube					
44. Undegraded	Guinea pig	1	≤ 1.5	20–30 days	Drink		FOB+	Multiple ulcerations of cecum; 80% had ulcerations. Crypt abscesses present; macrophages, with metachromatic material.		(*92*)
Degraded	Guinea pig	≤ 5	≤ 2	20–30 days	Drink		Diarrhea by 10 days, FOB+	100% had ulcerations; ulceration extended into distal colon and rectum.		
45. Degraded	Guinea pig	0.1–5		30 days–1 year	Drink		Weight loss in guinea pig and rabbit, not rat or mouse. Blood and mucous in stool.	Hemorrhagic and ulcerative lesions in cecum, colon, or rectum in all four species; crypt abscesses present.		(*93*)
	Rabbit				Drink					
	Rat				Drink					
	Mouse				Drink					

Abbreviations: AOM, azoxymethane; bw, body weight, CG,carrageenan; DCG, degraded carrageenan; DMH, dimethylhydrazine; FOB, fecal occult blood; ip, intraperitoneal; NMU, nitrosomethylurea; PEG, polyethylene glycol; SC, subcutaneous; SI, slight; tube, gastric intubation; UCG, undegraded carrageenan.

[a]Studies are associated with neoplastic changes, unlike studies predominantly demonstrating intestinal ulcerations. [b]Increased proliferation or neoplasm and carrageenan alone. [c]Ulcerations and carrageenan alone. [d]Neoplasms in which carrageenan promoted carcinogenesis. [e]Studies with uptake to lymph node or other site. [f]Study demonstrating breakdown to lower molecular weight. [g]Studies demonstrating ulcerations in rat using degraded carrageenan.

lesions were routinely produced with carrageenan concentrations of 0.1–1%, which is similar to the concentration in a variety of food products (*7,12–14*).

Grasso et al. (*83*) demonstrated pinpoint cecal and colonic ulcerations in guinea pigs and rabbits given 5% undegraded, as well as degraded, carrageenan in the diet for 3–5 weeks. Lesions were not observed in ferrets and squirrel monkeys given degraded carrageenan by gastric intubation (*83*). Other investigators have also observed ulcerations after exposure to either degraded or undegraded carrageenan (*75,88*). Engster and Abraham (*75*) observed ulceration of cecum in guinea pigs given ι-carrageenan of molecular weight 21,000–107,000, demonstrating ulcerations were also caused by higher molecular weight carrageenan. Cecal ulcerations were not found with exposures to κ or λ carrageenan of molecular weight varying from 8,500–314,000.

Investigators have noted that carrageenan-induced ulcerations of the colon are dose dependent and related to duration of exposure (*52,53,67,68,70,89,90*). Kitsukawa et al. (*52*) observed small epithelial ulcerations in guinea pigs who received carrageenan in their drinking fluid at two days. Olsen and Paulsen (*68*) observed cecal lesions after 24 hr and confluent ulcerations after 7 days in guinea pigs that ingested a 5% carrageenan solution. In rats, superficial erosions were observed at the anorectal junction at 24 hr after 10% dietary carrageenan (*70*); these extended more proximally over time. In 5 days of feeding with a 5% carrageenan solution, Jensen et al. (*62*) observed as many as 111 ulcerations/cm^2 over the mucosal surface of the cecum in the guinea pig.

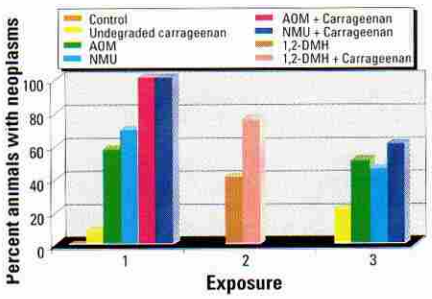

Figure 1. Carrageenan and promotion of neoplasms. No tumors were found in the control animals. With AOM and undegraded carrageenan, there was a 10-fold increase in the number of tumors per rat. See text for exposure regimens (*73*). 1,2-Dimethylhydrazine (1,2-DMH) alone caused neoplams in 40% of animals tested; with addition of undegraded carrageenan, 75% of exposed animals had tumors that were larger and occurred more frequently proximal (*57*). The combination of 1,2-DMH and degraded carrageenan was associated with an increase in small intestinal tumors from 20% to 50% of exposed animals and with an increase from 45% to 60% in large intestinal tumors (*64*).

Benitz et al. (*82*) observed a dose effect when degraded carrageenan was given at concentrations of 0.5–2% in drinking fluid to rhesus monkeys for 7–14 weeks. Watt and Marcus (*89*) observed that in rabbits given 0.1% degraded carrageenan as drinking fluid, 60% of the animals developed ulcerations, whereas 100% of those given 1% carrageenan had ulcerations when exposed for 6–12 weeks.

Resemblance to ulcerative colitis. Several investigators have noted the resemblance between the ulcerative lesions and accompanying inflammatory changes induced by carrageenan and the clinical spectrum of ulcerative colitis (*56,94–99*). Since the development of the carrageenan-induced model of ulcerative disease of the colon in 1969, carrageenan exposure has been used to model ulcerative colitis and to test for response to different treatments (*52,62,100,101*).

Clinical features in the experimental animals exposed to carrageenan have included weight loss, anemia, diarrhea, mucous in stools, and visible or occult blood in stools. The absence of small intestinal lesions and the lack of remission and exacerbation are also characteristic features of the carrageenan model (*99,102*).

Onderdonk (*94*) discussed the similarity between the carrageenan model of colitis and ulcerative colitis in humans and considered whether animal models for inflammatory bowel disease were also models for intestinal cancer because of the increased risk of colon cancer in individuals with ulcerative colitis. He reviewed the findings from carrageenan-treated animals, including loss of haustral folds, mucosal granularity, crypt abscesses, lymphocytic infiltration, capillary congestion, pseudopolyps, and strictures. Other observations have demonstrated an apparent sequence from colitis to squamous metaplasia and then to tumors of the colorectum (*67,72,102*). Atypical epithelial hyperplasia in the vicinity of carrageenan-induced ulcerations resembled findings from human ulcerative colitis that provide a link to intestinal neoplasia (*86,98*).

Proposed mechanism of development of lesions. A common feature observed in the animal models of ulceration in association with carrageenan exposure is macrophage

Table 4. Proposed mechanism for effects of carrageenan (*9,10,35,67,72,75,76,79,84–86,88,98,102,105,107–110,114,115*).

Site	Effect
Intestinal lumen	Ingested carrageenan can undergo acid hydrolysis in stomach, possible breakdown by intestinal bacteria.
Intestinal epithelial cells	Take up degraded carrageenan, as indicated by metachromatic staining from cecum to rectum. Vacuoles observed to contain metachromatic material. Epithelial cells may undergo lysis from effect of lysosomal disruption producing erosions.
Inflammatory infiltrate	Polymorphonuclear cells and macrophages infiltrate to site of intestinal inflammation. Macrophages have metachromatic staining associated with uptake of degraded carrageenan. Lysosomal vacuolation occurs as well as lysosomal disruption with release of intracellular enzymes from macrophage destruction, leading to intestinal ulcerations. Process of chronic inflammation, as with ulcerative colitis.
Macrophage circulation	Macrophages may circulate and may lead to extraintestinal effects related to carrageenan.

Table 5. Experimental evidence for presence of low molecular weight carrageenan in food-grade carrageenan and production of low molecular weight carrageenan by acid hydrolysis or by bacteria. (*9,10,36–40*).

Degraded carrageenan in food-grade carrageenan
25% of total carrageenans in eight food-grade κ-carrageenans had MW < 100,000
9% of total carrageenan in eight food-grade κ-carrageenans had MW < 50,000
Production of degraded carrageenan by acid hydrolysis of food-grade carrageenan
In simulated gastric fluid (including pepsin and HCL), κ-carrageenan at pH 1.2, 37°C
for 1 hr leads to 17% degraded carrageenan with MW < 20,000
for 2 hr leads to 25% with MW < 20,000
In simulated gastric fluid (including pepsin and HCL), κ-carrageenan at pH 1.9, 37°C
for 1 hr leads to 8% with MW < 20,000
for 2 hr leads to 10% with MW < 20,000
κ-carrageenan in solution at pH 1.0, 37°C, for 6 hours, leads to 25% with MW < 20,000
ι-carrageenan in solution at pH 1.0, 37°C, for 6 hours, leads to 10% with MW < 25,000
Hydrolysis of carrageenan by bacterial carrageenases
κ- and ι-carrageenase from cell-free supernatant from culture of *Cytophaga* genus
κ-carrageenase isolated from cell-free medium of cultured *Pseudomonas carrageenovora*
λ-carrageenase from cell-free medium of *Pseudomonas carrageenovora* cultures

MW, molecular weight.

infiltration (*35,56,63,65,68,75,76,78–81, 83,84,88,92,102–104*). Fibrillar material and metachromatic staining of the macrophages were observed. Notably, the macrophage lysosomes appeared to take up the fibrillar material and to become distorted and vacuolated. It appeared that colonic ulcerations developed as a result of macrophage lysosomal disruption, with release of intracellular enzymes, subsequent macrophage lysis, and release of intracellular contents that provoked epithelial ulceration (*75,76,79,84,85,88, 105,106*). In the rhesus monkey, Mankes and Abraham (*76*) observed vacuolated macrophages with fibrillar material when the animals were given undegraded carrageenan of molecular weight 800,000 as a 1% solution in their drinking fluid, demonstrating the occurrence of these changes after exposure to undegraded as well as to degraded carrageenan.

In an effort to clarify further the precise pathogenic changes that occurred, Marcus et al. (*35*) evaluated pre-ulcerative lesions after exposure of guinea pigs to degraded carrageenan for only 2–3 days. The animals received 3% drinking solution of carrageenan, with an average daily carrageenan intake of 5.8 g/kg. Early focal lesions were observed macroscopically in the cecum in only one animal with this brief exposure. However, in all test animals, a diffuse cellular infiltrate, with macrophages and polymorphonuclear leukocytes, was apparent microscopically. Inflammatory changes in the cecum and ascending colon were present in all animals, and in the distal colon and rectum in three of four animals. Metachromatic staining material was noted in the lamina propria of the colon and surface epithelial cells from cecum to rectum, as well as in colonic macrophages. The surface epithelial cells and the macrophages contained vacuoles filled with the metachromatic material, which was not found in the controls and not seen in more advanced lesions in previous studies. These early lesions suggested that the presence of degraded carrageenan within surface epithelial cells might be associated with the subsequent breakdown of the mucosa and to ulceration by a direct toxic effect on the epithelial cells (*35*).

Hence, a model of mechanical cellular destruction by disruption of lysosomes from carrageenan exposure arises from review of the experimental studies in animals. The observed changes in the lysosomes resemble the characteristic changes observed in some lysosomal storage diseases, in which there is accumulation of sulfated metabolites that cannot be processed further due to sulfatase enzyme deficiency (*107–110*). Table 4 presents a proposed mechanism of the effects of carrageenan.

Possible role of intestinal bacteria. The relationship between the intestinal microflora and the biologic activity of carrageenan has been reviewed (*111,112*). Investigators have examined the impact of antibiotics and alteration of the resident microbial flora on the activity of carrageenan. Grasso et al. (*83*) studied the impact of neomycin treatment on the development of ulcerations by carrageenan. Pretreatment against coliforms failed to attenuate the course of carrageenan-associated ulcerations (*80,83*). Pretreatment with metronidazole was effective in preventing carrageenan-induced colitis in another experiment, although there was no benefit in established colitis (*71*). Aminoglycosides administered after carrageenan exposure were associated with reduced mortality, but not with reduction in the number of colonic ulcerations (*94*). Hirono et al. (*65*) found increased ulcerations and squamous metaplasia from the anorectal junction to the distal colon in germ-free rats fed 10% carrageenan for less than 63 days.

Additional considerations about the mechanism of action of carrageenan involved the role of production of hydrogen sulfide gas from metabolism of carrageenan in the digestive tract. Because carrageenan is heavily sulfated (up to 40% by weight), bacterial sulfatases and sulfate reductases can produce hydrogen sulfide gas or HS^- from carrageenan. Carrageenan, as well as other sulfated polysaccharides, has been shown to stimulate H_2S production from fecal slurries (*113*). Sulfide has been implicated in the development of ulcerative colitis, perhaps attributable to interference with butyrate oxidation by colonic epithelial cells (*114,115*). Butyrate has been shown to induce intestinal cellular differentiation, suppress intestinal cell growth, and decrease expression of c-*myc*, among other functions in colonic epithelial cells (*116–118*).

No fermentation of carrageenan was reported after testing with 14 strains of intestinal bacteria. The increase in sulfide production observed arising from incubation of λ-carrageenan with colonic bacteria demonstrates that intestinal metabolism of carrageenan does occur. However, data pertaining to breakdown of carrageenan by fecal organisms are limited (*112,113*).

Extraintestinal manifestations of carrageenan exposure. Trace amounts of undegraded carrageenan have been reported to cross the intestinal barrier, with accumulation of label in intestinal lymph nodes (*61,74*). Several investigators have noted uptake of carrageenan by intestinal macrophages with subsequent migration of these macrophages to lymph nodes, spleen, and liver (*61,67,74,78,82,84,85*).

In association with carrageenan-induced intestinal ulcerations, Delahunty et al. (*56*) observed an increased permeability to large molecules, such as [^{3}H]PEG (polyethylene glycol)-900. This finding suggested that the intestinal changes induced by carrageenan may be a factor in subsequent absorption of carrageenan or other large molecules.

Other experimental data. Because it can induce acute inflammation, carrageenan has been widely used in experimental models of inflammation to assess activity of anti-inflammatory drugs and to study mediators of inflammation (*4,61,106,119,120*). Injected into an experimental site, such as the plantar surface of a rat's paw, pleural cavity, or subcutaneous air bleb, carrageenan induces an inflammatory response, with edema, migration of inflammatory cells, predominantly polymorphonuclear leukocytes, and possibly granuloma formation (*61,120*). Undegraded carrageenans *in vitro* can inhibit binding of basic fibroblast growth factor (bFGF), transforming growth factor β-1, and platelet-derived growth factor but not insulin-like growth factor-1 or transforming growth factor-α (*121*).

Macrophage injury and destruction caused by carrageenan may be a factor in the reduced cytotoxic lymphocytic response associated with carrageenan exposure *in vivo* (*122*). In addition to depression of cell-mediated immunity, impairment of complement activity and of humoral responses have been reported. Prolongation of graft survival and potentiation of tumor growth have been attributed to the cytopathic effect on macrophages (*96,123*). Because of its effect on T-cells, carrageenan has been studied for its impact on viral infections with herpes simplex virus types 1 and 2 (*124*) and HIV-1 (*125,126*), as well as infections with *Chlamydia trachomatis* (*127*).

In experimental systems, undegraded carrageenan has produced destruction of several different cell types in addition to macrophages, including small intestine epithelial cell monolayers (*54*), androgen-dependent malignant prostatic cells (*128*), bFGF-dependent endothelial cell line (*128*), rat mammary adenocarcinoma 13762 MAT cells (*129*), and human mammary myoepithelial cells (*130*). Lysosomal inclusions and vacuolation have been observed in macrophages, intestinal epithelial cells, and myoepithelial cells exposed to carrageenan (*79,85,131*).

Injections of carrageenan were noted to induce sarcomas, as well as mammary tumors in animal models, in an early study (*132*). In other experiments, mammary and testicular tumors have been observed (*69,133*). Carrageenan has also been noted to have anticoagulant activity, and large systemic doses have been fatal through nephrotoxicity (*4*).

Mechanisms for Production of Degraded Carrageenan from Undegraded Carrageenan

Gastrointestinal metabolism of carrageenan to form smaller molecular weight components has been observed by several investigators, who reported that carrageenan of high molecular weight changed during intestinal passage, compatible with hydrolysis yielding lower molecular weight components (*9,10,74,75*).

Under conditions such as might occur in digestion, 17% of food-grade carrageenan degraded to molecular weight < 20,000 in 1 hr at pH 1.2 at 37°C. At pH 1.9 for 2 hr at 37°C, 10% of the carrageenan had molecular weight less than 20,000 (*9*). These data suggest that substantial fractions of lower molecular weight carrageenan are likely to arise during normal digestion.

Table 5 presents data with regard to contamination of food-grade carrageenan by lower molecular weight carrageenan. Twenty-five percent of total carrageenans in eight food-grade carrageenans were found to have molecular weight < 100,000, with 9% having molecular weight < 50,000 (*9*). In addition, several bacteria have been identified that are able to hydrolyze carrageenan into smaller products, including tetracarrabiose. These bacteria, including *Cytophaga* species and *Pseudomonas carrageenovora*, are of marine origin; it is unknown whether the human microbial flora can perform similar hydrolysis reactions (*36–40,134*).

Extent of Human Exposure to Carrageenan

Indirect evidence relating exposure to carrageenan and the occurrence of ulcerative colitis and intestinal neoplasms consists of the similar geographic distribution between higher consumption of carrageenan and higher incidence of inflammatory bowel disease and colorectal cancer. Ulcerative colitis is more common in North America, the United Kingdom, and Scandinavia, and less common in Central and Southern Europe, Asia, and Africa (*135*). This incidence distribution is similar to distributions for colorectal malignancy and for carrageenan consumption, providing some ecologic evidence to support a potential etiologic role of carrageenan in human disease (*46,136*).

The reported TD_{50} (tumorigenic dose 50% = the dose rate, in milligrams per kilogram body weight per day, which will halve the probability of remaining tumorless over the life span of the exposed animal) by the Carcinogenic Potency Database for degraded carrageenan is 2,310 mg/kg body weight/day, based on rodent experiments (*137,138*). This extrapolates to 138.6 grams for a 60-kg individual. If the total carrageenan intake per person in the United States is about 100 mg a day (*43*), about 9 mg of carrageenan with molecular weight < 50,000 is likely to be ingested through contamination of food-grade carrageenan by degraded carrageenan, and at least 8 mg with molecular weight < 20,000 is likely to arise during normal digestion (simulated by exposure to pH 1.9 with pepsin for 1 hr at 37°C). This suggests an average intake of about 10 mg/day of degraded carrageenan for an individual older than 2 years of age in the United States.

An important issue is whether 10 mg/day degraded carrageenan is safe to ingest. By the Delaney clause, no carcinogen should be permitted in food. The Food Quality Protection Act (FQPA) established a usage level for negligible risk associated with pesticide residue in food at 1 ppm (*139,140*). Applying this standard to the extrapolated TD_{50} for degraded carrageenan for a 60-kg person, the anticipated average intake of 10 mg/day is 70-fold greater than this standard (138.6 g/10^6/day). These calculations do not take into consideration possible exposure to furcellaran (molecular weight 20,000–80,000), or the wide range of possible intakes of carrageenan.

Conclusion

Inflammatory bowel disease and colorectal malignancy represent major sources of morbidity and mortality in the United States. A possible factor in the etiology of these pathologies is exposure to carrageenan.

Several investigators have expressed their concerns about the use of undegraded carrageenan in food products (*6–10*), yet no legislative protection to restrict incorporation of low molecular weight fractions has been enacted. In fact, there has been no substantive review by the Food and Drug Administration of carrageenan since the studies undertaken more than two decades ago. However, there has been increased evidence regarding the cancer-promoting activity of undegraded carrageenan and further confirmation of the carcinogenic potential of degraded carrageenan.

Evidence for the role in carcinogenesis of carrageenan appears to support a nongenotoxic model based on direct toxic effects, for carrageenan has been nonmutagenic in *Salmonella* mutagenicity testing and nongenotoxic by DNA repair tests (*60,102*). A model of cellular destruction—from disruption of lysosomes by accumulation of carrageenan by-products or by interference with normal cellular oxidation–reduction processes from sulfate metabolites—emerges from review of the experimental studies. The impact of sulfatases, of either bacterial or human origin, on the metabolism of carrageenan requires further investigation. By interference with the normal intracellular feedback mechanisms associated with arylsulfatase activity, including steroid sulfatase, the highly sulfated carrageenan may have an impact on the availability of active, unsulfated hormones, such as dehydroepiandrosterone, derived from dehydroepiandrosterone-sulfate, and estrone-1, derived from estrone-1 sulfate.

Genetic characteristics that affect sulfatase and hydrolysis reactions as well as the individual intestinal microflora may influence how carrageenan is metabolized and how its effects are manifested. These factors may determine how carrageenan is metabolized differently by different individuals, but these characteristics may not be accessible to manipulation. A basic factor that can be controlled is the intake of carrageenan, which is amenable to dietary modification or food additive regulation.

Although carrageenan is widely used as a food additive for its texture-enhancing properties, other gums, some of which are used in combination with carrageenan, such as locust bean gum, gum arabic, alginate, guar gum, or xanthan gum, potentially can be used alone or in different combinations as substitutes for carrageenan (*41,46*). Alternatively, higher fat composition can lead to changes in food properties that may compensate for exclusion of carrageenan. Other hydrocolloids that are used as stabilizers and thickeners have not been associated with harmful gastrointestinal effects, and it is reasonable to expect that they could replace carrageenan in many food products. Although the dietary fibers pectin and psyllium affect intestinal motility, ulcerations or neoplasms have not been induced with either these or the other water-soluble polymers used as food additives. In contrast, other highly sulfated polysaccharides, amylopectin sulfate and dextran sulfate sodium, have induced ulcerations and neoplasia, suggesting that the degree of sulfation and polysaccharide molecular weight may be critical for induction of the observed effects (*102*).

The major pieces of evidence that support an argument to reconsider the advisability of use of carrageenan as a GRAS food additive are:

- Degraded carrageenan is a known carcinogen in animal models
- Undegraded carrageenan is a known co-carcinogen in animal models of carcinogenesis
- In animal models, both degraded and undegraded carrageenan have been associated with development of intestinal ulcerations that resemble ulcerative colitis
- Hydrolysis such as may occur by exposure to gastric acid in the human stomach can lead to the depolymerization of undegraded carrageenan and the availability of degraded carrageenan
- Food-grade carrageenan may be contaminated with low molecular weight, degraded

carrageenan that may arise during food processing

- The use of a viscosity measurement to characterize a carrageenan sample is insufficient because the presence of a small number of large molecules (and undegraded carrageenan may have molecular weight in the millions) may obscure a significant low molecular weight fraction.

The potential role of carrageenan in the development of gastrointestinal malignancy and inflammatory bowel disease requires careful reconsideration of the advisability of its continued use as a food additive.

References and Notes

1. Ries LAG, Kosary CL, Hankey BF, Miller BA, Clegg L, Edwards BK. SEER Cancer Statistics Review 1973–1996. Bethesda, MD:National Cancer Institute, 1999.
2. Schottenfeld D, Winawer SJ. Cancers of the large intestine. In: Cancer Epidemiology and Prevention (Schottenfeld D, Fraumeni J, eds). 2nd ed. New York:Oxford University Press, 1996;813–840.
3. Schatzkin A. Available: http://rex.nci.nih.gov/NCI_Pub_Interface/raterisk/risks129.html [cited 6 October 2000].
4. IARC. IARC Working Group on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Carrageenan. IARC Monogr Eval Carcinog Risk Hum 31:79–94 (1983).
5. National Research Council. Carcinogens and Anti-carcinogens in the Human Diet. Washington, DC:National Academy Press, 1996;398.
6. Marcus R, Watt J. Danger of carrageenan in foods and [Letter]. Lancet 1:338 (1981).
7. Marcus R, Watt J. Potential hazards of carrageenan [Letter]. Lancet 1:602–603 (1980).
8. Marcus R. Harmful effects of carrageenan fed to animals. Cancer Detect Prev 4:129–134 (1981).
9. Ekstrom L-G. Molecular weight distribution and the behavior of kappa-carrageenan on hydrolysis. Carbohydr Res 135:283–289 (1985).
10. Ekstrom L-G, Kuivinen J, Johansson G. Molecular weight distribution and hydrolysis behavior of carrageenans. Carbohydr Res 116:89–94 (1983).
11. Yu G, Ioanoviciu AS, Sikkander SA, Thanawiroon C, Toida T, Tobacman J, Linhardt RJ. Unpublished data.
12. Klose RE, Glicksman M. Gums. In: Handbook of Food Additives (Furia TE, ed). Cleveland, OH:The Chemical Rubber Co., 1968;313–375.
13. Towle GA. Carrageenan. In: Industrial Gums: Polysaccharides and Their Derivatives (Whistler RL, ed). New York:Academic Press, Inc., 1973;84–109.
14. Moirano AL. Sulfated seaweed polysaccharides. In: Food Colloids (Graham HD, ed). Westport, CT:AVI Publishing Co., 1977;347–381.
15. Daniel JR, Voragen ACJ, Pilnik W. Starch and other polysaccharides. In: Ullmann's Encyclopedia of Industrial Chemistry, Vol A 25 (Elvers B, Hawkins S, Russey W, eds). New York:VCH Verlagsgesellschaft, 1994;21–62.
16. Substances that are generally recognized as safe. Fed Reg 21:9368–9370.
17. Food and Drugs: Food Additives. 21 C.F.R. 121.101,121.1063,121.1066,121.1067,121.1069, 1969.
18. Proposed Revision of Food Additive Regulations and Deletion of Chondrus Extract (Carrageenin) from Generally Regarded as Safe (GRAS) List. 37 Fed Reg 15434.
19. Informatics, Inc. Carrageenan. Arlington, VA:National Technical and Information Service, 1972;1–68.
20. Nicklin S, Miller K. Intestinal uptake and immunological effects of carrageenan—current concepts. Food Addit Contam 6(4):425–436 (1989).
21. Food and Nutrition Board, National Research Council. Estimating Distribution of Daily Intakes of *Chondrus* Extract (Carrageenan): Committee on GRAS List Survey–Phase III. Appendix C. Washington, DC:National Academy of Sciences, 1976;1–7.
22. Stanicoff DJ, Renn DW. Physiological effects of carrageenan. In: ACS Symposium Series (15): Physiological Effects of Food Carbohydrates (Gould RF, ed). Washington, DC:American Chemical Society, 1975; 282–295.
23. Pintauro SJ, Gilbert SW. The effects of carrageenan on drug-metabolizing enzyme system activities in the guinea-pig. Food Chem Toxicol 28:807–811 (1990).
24. Carrageenan, Salts of Carrageenan and Chondrus Extract (Carrageenin); Withdrawal of Proposal and Termination of Rulemaking Proceeding. Fed Reg 44:40343–40345.
25. International Food Additives Council and FMC Corporation-Marine Colloids Division, filing of Food Additive Petitions; Hercules, Inc.; Notice of Receipt of Citizen Petition; Request for Comments. Fed Reg 57:49483–49485.
26. National Research Council. Food Chemical Codex. 2nd ed, suppl 2. Washington, DC:National Academy of Science, 1975.
27. National Research Council. Food Chemical Codex. 4th ed. Washington, DC:National Academy of Science, 1996.
28. Tong H-K, Lee K-H, Wong H-A. The molecular weight and viscosity of the water-soluble polysaccharide(s) from *Eucheuma spinosum*. Carbohydr Res 81:1–6 (1980).
29. Weiner ML. Toxicological properties of carrageenan. Agents Actions 32(1/2):46–51 (1991).
30. Food and Drugs: Food Additives Permitted for Direct Addition to Food for Human Consumption. 21 C.F.R. 172.620,172.626,172.655,172.660, 2000.
31. Food and Drugs: Substances Generally Regarded as Safe. 21 C.F.R. 182.7255, 1999.
32. Food and Drugs: New Drugs. 21 C.F.R. 310.545, 1999.
33. Food and Drugs: 21 C.F.R. 133.178, 133.179, 136.110, 139.121, 139.121, 139.122, 150.141, 150.161, 176.170 (2000).
34. Watt J, McLean C, Marcus R. Degradation of carrageenan for the experimental production of ulcers in the colon. J Pharm Pharmacol 31:645–646 (1979).
35. Marcus SN, Marcus AJ, Marcus R, Ewen SWB, Watt J. The pre-ulcerative phase of carrageenan-induced colonic ulceration in the guinea-pig. Int J Exp Pathol 73:515–526 (1992).
36. Sarwar G, Matoyoshi S, Oda H. Purification of a κ-carrageenase from marine *cytophaga* species. Microbiol Immunol 31:869–877 (1987).
37. Weigl J, Yaphe W. The enzymic hydrolysis of carrageenan by *pseudomonas carrageenovora*: purification of a κ-carrageenase. Can J Microbiol 12:939–947 (1986).
38. Potin P, Sanseau A, LeGall Y, Rochas C, Bloareg B. Purification and characterization of a new κ-carrageenase from a marine *cytophaga*-like bacterium. Eur J Biochem 201:241–247 (1991).
39. McLean MW, Williamson FB. κ-Carrageenase from *Pseudomonas carrageenovora*. Eur J Biochem 93:553–558 (1979).
40. Johnston KH, McCandless EL. Enzymic hydrolysis of the potassium chloride soluble fraction of carrageenan: properties of "lambda carrageenases" from *Pseudomonas carrageenovora*. Can J Microbiol 19(7):779–788 (1973).
41. Friedman LJ, Greenwald CG. Food additives. In: Encyclopedia of Chemical Technology, Vol 11 (Howe-Grant M, ed). 4th ed. New York:John Wiley & Sons, 1994;805–833.
42. Meer WA. Plant hydrocolloids. In: Food Colloids (Graham HD, ed). Westport, CT:AVI Publishing Company, Inc., 1977;522–539.
43. Food and Nutrition Board, National Research Council. The 1977 Survey of Industry on the Use of Food Additives: Committee on GRAS List Survey–Phase III. Part 3. PB 80–113418. Washington, DC:National Academy of Sciences, 1979.
44. Anderson W. Carrageenan: structure and biological activity. Can J Pharm Sci 2:81–90 (1967).
45. Comité "Additifs Alimentaires" du CNERNA. Toxicological evaluation of carrageenans. 10-Conclusions: acquired knowledges and problems requiring further researches. Sciences des aliments 4:429–438.
46. Will R, Zuanich J, DeBoo A, Ishikawa Y. Water-soluble polymers. Menlo Park, CA:Chemical Economics Handbook - SRI International, 1999;582.0000E–582.0003V.
47. Piculell L. Gelling carrageenans. In: Food Polysaccharides and Their Applications. New York:Marcel Dekker, Inc., 1995;205–244.
48. Food and Nutrition Board, National Research Council. 1977 Survey of Industry on the Use of Food Additives. Summarized Data: Committee on GRAS List Survey–Phase III. Washington, DC:National Academy of Sciences, 1979;978–987.
49. Food Protection Committee, Food and Nutrition Board, National Research Council. Chemicals Used in Food Processing. Publication 1274. Washington, DC:National Academy of Sciences, 1965;31–34.
50. Corpet DE, Taché S, Préclaire M. Carrageenan given as a jelly, does not initiate, but promotes the growth of aberrant crypt foci in the rat colon. Cancer Lett 114:53–55 (1997).
51. Wilcox DK, Higgins J, Bertram TA. Colonic epithelial cell proliferation in a rat model of nongenotoxin-induced colonic neoplasia. Lab Invest 67:405–411 (1992).
52. Kitsukawa Y, Saito H, Suzuki Y, Kasanuki J, Tamura Y, Yoshida S. Effect of ingestion of eicosapentaenoic acid ethyl ester on carrageenan-induced colitis in guinea pigs. Gastroenterology 102:1859–1866 (1992).
53. Marcus AJ, Marcus SN, Marcus R, Watt J. Rapid production of ulcerative disease of the colon in newly-weaned guinea-pigs by degraded carrageenan. J Pharm Pharmacol 41:423–426 (1989).
54. Ling K-Y, Bhalla D, Hollander D. Mechanisms of carrageenan injury of IEC18 small intestinal epithelial cell monolayers. Gastroenterology 95:1487–1495 (1988).
55. Calvert RJ, Reicks M. Alterations in colonic thymidine kinase enzyme activity induced by consumption of various dietary fibers. Proc Soc Exp Biol Med 189:45–51 (1988).
56. Delahunty T, Recher L, Hollander D. Intestinal permeability changes in rodents: a possible mechanism for degraded carrageenan-induced colitis. Food Chem Toxicol 25:113–118 (1987).
57. Arakawa S, Okumua M, Yamada S, Ito M, Tejima S. Enhancing effect of carrageenan on the induction of rat colonic tumors by 1,2-dimethylhydrazine and its relation to β-glucuronidase activities in feces and other tissues. J Nutr Sci Vitaminol (Tokyo) 32:481–485 (1986).
58. Kitano A, Matsumoto T, Hiki M, Hashimura H, Yoshiyasu K, Okawa K, Kuwajima S, Kobayashi K. Epithelial dysplasia of the rabbit colon induced by degraded carrageenan. Cancer Res 46:1374–1376 (1986).
59. Fath RB, Deschner EE, Winawer SJ, Dworkin BM. Degraded carrageenan-induced colitis in CF_1 mice. Digestion 29:197–203 (1984).
60. Mori H, Ohbayashi F, Hirono I, Shimada T, Williams GM. Absence of genotoxicity of the carcinogenic sulfated polysaccharide carrageenan and dextran sulfate in mammalian DNA repair and bacterial mutagenicity assays. Nutr Cancer 6:92–97 (1984).
61. Nicklin S, Miller K. Effect of orally administered food-grade carrageenans on antibody-mediated and cell-mediated immunity in the inbred rat. Food Chem Toxicol 22:615–621 (1984).
62. Jensen BH, Andersen JO, Poulsen SS, Olsen PS, Rasmussen SN, Hansen SH, Hvidberg DF. The prophylactic effect of 5-aminosalicylic acid and salazosulphapyridine on degraded-carrageenan-induced colitis in guinea pigs. Scand J Gastroenterol 19:299–303 (1984).
63. Olsen PS, Kirkegaard P, Poulsen SS. The effect of ileo-transversostomy on carrageenan-induced colitis in guinea pig. Scand J Gastroenterol 18:407–410 (1983).
64. Kawaura A, Shibata M, Togei K, Otsuka H. Effect of dietary degraded carrageenan on intestinal carcinogenesis in rats treated with 1,2-dimethylhydrazine dihydrochloride. Tokushima J Exp Med 29:125–129 (1982).
65. Hirono I, Sumi Y, Kuhara K, Miyakawa M. Effect of degraded carrageenan on the intestine in germfree rats. Toxicol Lett 8:207–212 (1981).
66. Norris AA, Lewis AJ, Zeitlin IJ. Inability of degraded carrageenan fractions to induce inflammatory bowel ulceration in the guinea pig. J Pharm Pharmacol 33:612–613 (1981).
67. Oohashi Y, Ishioka TT, Wakabayashi K, Kuwabara N. A study of carcinogenesis induced by degraded carrageenan arising from squamous metaplasia of the rat colorectum. Cancer Lett 14:267–272 (1981).
68. Olsen PS, Poulsen SS. Stereomicroscopic and histologic changes in the colon of guinea pigs fed degraded carrageenan. Acta Pathol Microbiol Scand Sect A 88:135–141 (1980).
69. Rustia M, Shubik P, Patil K. Lifespan carcinogenicity tests with native carrageenan in rats and hamsters. Cancer Lett 11:1–10 (1980).
70. Oohashi Y, Kitamura S, Wakabayashi K, Kuwabara N, Fukuda Y. Irreversibility of degraded carrageenan-induced colorectal squamous metaplasia in rats. Gann 70:391–392 (1979).

71. Onderdonk AB, Hermos JA, Dzink JL, Bartlett JG. Protective effect of metronidazole in experimental ulcerative colitis. Gastroenterology 74:521–526 (1978).
72. Wakabayashi K, Inagaki T, Fujimoto Y, Fukuda Y. Induction by degraded carrageenan of colorectal tumors in rats. Cancer Lett 4:171–176 (1978).
73. Watanabe K, Reddy BS, Wong CQ, Weisburger JH. Effect of dietary undegraded carrageenan on colon carcinogenesis in F344 rats treated with azoxymethane or methylnitrosourea. Cancer Res 38:4427–4430 (1978).
74. Pittman KA, Golberg L, Coulston F. Carrageenan: the effect of molecular weight and polymer type on its uptake, excretion and degradation in animals. Food Cosmet Toxicol 14:85–93 (1976).
75. Engster M, Abraham R. Cecal response to different molecular weights and types of carrageenan in the guinea pig. Toxicol Appl Pharmacol 38:265–282 (1976).
76. Mankes R, Abraham R. Lysosomal dysfunction in colonic submucosal macrophages of rhesus monkeys caused by degraded iota carrageenan. Proc Soc Exp Biol Med 150:166–170 (1975).
77. Iatropoulos MJ, Golberg L, Coulston L. Intestinal carcinogenesis in rats using 1,2-dimethylhydrazine with or without degraded carrageenan. Exp Mol Pathol 23:386–401 (1975).
78. Grasso P, Gangolli SD, Butterworth KR, Wright MG. Studies on degraded carrageenan in rats and guinea-pigs. Food Cosmet Toxicol 13:195–201 (1975).
79. Abraham R, Fabian RJ, Golberg MB, Coulston F. Role of lysosomes in carrageenan-induced cecal ulceration. Gastroenterology 67:1169–1181 (1974).
80. Van der Waaif D, Cohen BJ, Anver MR. Mitigation of experimental inflammatory bowel disease in guinea pigs by selective elimination of the aerobic gram-negative intestinal microflora. Gastroenterology 67:460–472 (1974).
81. Poulsen E. Short-term peroral toxicity of undegraded carrageenan in pigs. Food Cosmet Toxicol 11:219–227 (1973).
82. Benitz K-F, Golberg L, Coulston F. Intestinal effects of carrageenans in the rhesus monkey. Food Cosmet Toxicol 11:565–575 (1973).
83. Grasso P, Sharratt M, Carpanini FMB, Gangolli SD. Studies on carrageenan and large-bowel ulceration in mammals. Food Cosmet Toxicol 11:555–564 (1973).
84. Fabian RJ, Abraham R, Coulston F, Golberg L. Carrageenan-induced squamous metaplasia of the rectal mucosa in the rat. Gastroenterology 65:265–276 (1973).
85. Abraham R, Golberg L, Coulston F. Uptake and storage of degraded carrageenan in lysosomes of reticuloendothelial cells of the rhesus monkey. Exp Mol Pathol 17:77–93 (1972).
86. Watt J, Marcus R. Carrageenan-induced ulceration of the large intestine in the guinea pig. Gut 12:164–171 (1971).
87. Marcus R, Watt J. Colonic ulceration in young rats fed degraded carrageenan. Lancet 2:765–766 (1971).
88. Sharratt M, Grasso P, Carpanini F, Gangolli SD. Carrageenan ulceration as a model for human ulcerative colitis. Lancet 2:932 (1970).
89. Watt J, Marcus R. Ulcerative colitis in rabbits fed degraded carrageenan. J Pharm Pharmacol 22:130–131 (1970).
90. Watt J, Marcus R. Hyperplastic mucosal changes in the rabbit colon produced by degraded carrageenin. Gastroenterology 59:760–768 (1970).
91. Maillet M, Bonfils S, Lister RE. Carrageenan: effects in animals. Lancet 2:414–415 (1970).
92. Watt J, Marcus R. Ulcerative colitis in the guinea-pig caused by seaweed extract. J Pharm Pharmacol 21:187S–188S (1969).
93. Marcus R, Watt J. Seaweeds and ulcerative colitis in laboratory animals. Lancet 2:489–490 (1969).
94. Onderdonk AB. The carrageenan model for experimental ulcerative colitis. Prog Clin Biol Res 186:237–245 (1985).
95. Ottet NK. On animal models for inflammatory bowel disease. Gastroenterology 62:1269–1272 (1972).
96. Watt J, Marcus R. Progress report: Experimental ulcerative disease of the colon in animals. Gut 14:506–510 (1973).
97. Sharratt M, Grasso P, Carpanini F, Gangolli SD. Carrageenan ulceration as a model for human ulcerative colitis. Lancet 1:192–193 (1971).
98. Mottet NK. On animal models for inflammatory bowel disease. Gastroenterology 62:1269–1271 (1971).
99. Kim H-S, Berstad A. Experimental colitis in animal models. Scand J Gastroenterol 27:529–537 (1992).
100. Watt J, Marcus SN, Marcus AJ. The comparative prophylactic effects of sulfasalazine, prednisolone, and azathioprine in experimental ulceration. J Pharm Pharmacol 32:873–874 (1980).
101. Kitano A, Matsumoto T, Oshitani N, Nakagawa M, Yasuda K, Watanabe Y, Tomobuchi M, Obayashi M, Tabata A, Fukushima R, et al. Distribution and anti-inflammatory effect of mesalazine on carrageenan-induced colitis in the rabbit. Clin Exp Pharmacol Physiol 23:305–309 (1996).
102. Ishioka T, Kuwabara N, Oohashi Y, Wakabayashi K. Induction of colorectal tumors in rats by sulfated polysaccharides. CRC Crit Rev Toxicol 17:215–244 (1987).
103. Gangolli SD, Wright MG, Grasso P. Identification of carrageenan in mammalian tissues: an analytical and histochemical study. Histochem J 5:37–48 (1973).
104. Pipy B. 9-Carraghénanes et macrophages. Sciences des aliments 4:415–428 (1984).
105. Catanzaro PJ, Schwartz HJ, Graham RD. Spectrum and possible mechanism of carrageenan cytotoxicity. Am J Pathol 64:387–404 (1971).
106. Thomson AW, Fowler EF. Carrageenan: a review of its effect on the immune system. Agents Actions 1:265–273 (1981).
107. Kolodny EW, Fluharty AL. Metachromatic leukodystrophy and multiple sulfatase deficiency: sulfatide lipidosis. In: The Metabolic and Molecular Bases of Inherited Diseases (Scriver CR, AL Beaudet AL, Sly WS, Valle D, eds). 7th ed. New York: McGraw-Hill, Inc., 1995; 2693–2739.
108. Ballabio A, Shapiro LJ. Steroid sulfatase deficiency and X-linked ichthyosis. In: The Metabolic and Molecular Bases of Inherited Diseases (Scriver CR, Beaudet AL, Sly WS, Valle D, eds) 7th ed. New York: McGraw-Hill, Inc., 1995;2999–3022.
109. Cotran RS, Kumar V, Robbins SL, Schoen FJ. Genetic diseases. Robbins' Pathological Basis of Disease. 5th ed. Philadelphia:W.B. Saunders Company, 1994;123–171.
110. Muenzer J. Mucopolysaccharidoses. Adv Pediatr 33:269–302 (1986).
111. Corpet DE. Toxicological evaluation of carrageenans. 5-Dietary carrageenans and intestinal microflora. Sciences des aliments 4:367–374 (1984).
112. Michel C, Macfarlane GT. Digestive fates of soluble polysaccharides from marine macroalgae: involvement of the colonic microflora and physiological consequences for the host. J Appl Bacteriol 1996;80:349–369 (1996).
113. Gibson GR, Macfarlane S, Cummings JH. The fermentability of polysaccharides by mixed human faecal bacteria in relation to their suitability as bulk-forming laxatives. Lett Appl Microbiol 11:251–254 (1990).
114. Roediger WEW, Duncan A, Kapaniris O, Millard S. Reducing sulfur compounds of the colon impair colonocytes nutrition: implications for ulcerative colitis. Gastroenterology 104:802–809 (1993).
115. Richardson CJ, Magee EAM, Cummings JH. A new method for the determination of sulphide in gastrointestinal contents and whole blood by microdistillation and ion chromatography. Clin Chim Acta 293:115–125 (2000).
116. Babidge W, Millard S, Roediger W. Sulfides impair short chain fatty acid beta-oxidation at acyl-CoA dehydrogenase level in colonocytes: implications for ulcerative colitis. Mol Cell Biochem 181:117–124 (1998).
117. Toscani A, Soprano DR, Soprano KJ. Molecular analysis of sodium butyrate-induced growth arrest. Oncogene Res 3:223–238 (1998).
118. Glinghammar B, Holmberg K, Rafter J. Effects of colonic lumenal components on AP-1 dependent gene transcription in cultured human colon carcinoma cells. Carcinogenesis 20:969–976 (1999).
119. Salyers AA, West SHE, Vercelotti JR, Wilkins TD. Fermentation of mucins and plant polysacchairds by anerobic bacteria from the human colon. Appl Environ Microbiol 334:529–533 (1977).
120. Di Rosa M. Review: Biological properties of carrageenan. J Pharm Pharmacol 24:89–102 (1972).
121. Hoffman R. Carrageenans inhibit growth-factor binding. Biochem J 289:331–334 (1993).
122. Cochran FR, Baxter CS. Macrophage-mediated suppression of T-lymphocyte proliferation induced by oral carrageenan administration. Immunology 53:221–227 (1984).
123. Thomson AW, Fowler EF. Potentiation of tumor growth by carrageenan. Transplantation 24:397–400 (1977).
124. Carlucci MJ, Pujol CA, Ciancia M, Noseda MD, Matulewicz MC, Damonte EB, Cerezo AS. Antiherpetic and anticoagulant properties of carrageenans from the red seaweed *Gigartina skottsbergii* and their cyclized derivatives: correlation between structure and biological activity. Int J Biol Macromol 20:97–105 (1997).
125. Yamada T, Ogano A, Saito T, Watanabe J, Uchiyama H, Nakagawa Y. Preparation and anti-HIV activity of low-molecular-weight carrageenans and their sulfated derivatives. Carbohydr Polym 32:51–55 (1997).
126. Pearce-Pratt R, Phillips DM. Sulfated polysaccharides inhibit lymphocyte-to-epithelial transmission of human immunodeficiency virus-1. Biol Reprod 54:173–182 (1996).
127. Zaretzky FR, Pearce-Pratt R, Phillips DM. Sulfated polyanions block *Chlamydia trachomatis* infection of cervix-derived human epithelia. Infect Immun 63:3520–3526 (1995).
128. Hoffman R, Burns WW, Paper DH. Selective inhibition of cell proliferation and DNA synthesis by the polysulphated carbohydrate ι-carrageenan. Cancer Chemother Pharmacol 36:325–334 (1995).
129. Coombe DR, Parish CR, Ramshaw IA, Snowden JM. Analysis of the inhibition of tumour metastasis by sulphated polysaccharides. Int J Cancer 39:82–88 (1987).
130. Tobacman JK. Filament disassembly and loss of mammary myoepithelial cells after exposure to lambda-carrageenan. Cancer Res 57:2823–2826 (1997).
131. Tobacman JK, Walters K. Carrageenan exposure leads to mammary myoepithelial cell development of unusual intracellular inclusions. Proc Am Assoc Cancer Res 39:4722 (1999).
132. Cater DB. The carcinogenic action of carrageenin in rats. Br J Cancer 15:607–614 (1961).
133. Hopkins J. Carcinogenicity of carrageenan. Food Cosmet Toxicol 19:779–788 (1981).
134. Dyrset N, Lystad KQ, Levine DW. Development of a fermentation process for production of a kappa-carrageenase from *Pseudomonas carrageenovora*. Enzyme Microb Technol 20(6):418–423 (1997).
135. Irvine EJ, Farrokhyar F, Swarbrick ET. A critical review of epidemiological studies in inflammatory bowel disease. Scand J Gastroenterol 36(1):2–15 (2001).
136. Ferlay J, Bray F, Pisani P, Parkin DM. GLOBOCAN 2000: Cancer Incidence, Mortality and Prevalence Worldwide, Version 1.0. IARCCancerBase No. 5. Lyon:IARC Press, 2001. Limited version available: http://www-dep.iarc.fr/cgi-bin/exe-globom.exe [cited 2 March 2001].
137. Gold LS, Slone TH, Manley NB, Garfinkel GB, Rohrbach L, Ames BN. Carcinogenic potency database. In: Handbook of Carcinogenic Potency and Genotoxicity Databases (Gold LS, Zeiger E, eds). New York:CRC Press, Inc., 1997;116–117.
138. Gold LS, Slone TH, Ames BN. Summary of carcingogenic potency database by chemical. In: Handbook of Carcinogenic Potency and Genotoxicity Databases (Gold LS, Zeiger E, eds). New York:CRC Press, Inc., 1997;629.
139. Food Additives Amendment of 1958. Public Law 85–929, 72 Stat. 1784.
140. Food Quality Protection Act of 1996. Public Law 104–170, 110 Stat. 1489.