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. Environ Health Perspect 109:983–994 (2001). [Online ______] 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 Chemical 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 5000 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...
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 anhydログalactose; 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 dieticetic 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

| Chemical composition | Hydrocolloid composed of α-1,3 and β-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; ω and σ form right-handed helices; potassium chloride promotes gel formation of κ; calcium ion promotes gel formation of λ. |
| Metabolism           | Hydrolysis of glycosidic linkages at lower pH, especially pH ≤ 3.0; also desulfation by sulfatas. |
| 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 × 10^6 to 2 × 10^6; 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 | 0.005–0.2% by weight. |
| Major uses           | Milk products, processed meats, dieticetic formulations, infant formula, toothpaste, cosmetics, skin preparations, pesticides, laxatives. |
in Table 2. Because manufacturing practices vary and change over time and the food formulations 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 tables 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 τ 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 adenosmas. 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 polypoid 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). Ohashi 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 degraded 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...
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% \( \lambda \)-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 | Type of carrageenan, molecular weight | Animal | %CG (g/kg bw/day) | Dose (mg/kg ip) | Duration | Route | Additional exposure | Digestive/systemic | Histopathologic changes | Neoplastic changes | Reference |
|------------|--------------------------------------|--------|-------------------|----------------|----------|-------|---------------------|-------------------|------------------------|---------------------|-----------|
| 1. Undegraded \( \alpha \) | Rat | 10 | 27.4 | 8 days | AOM injection | Less weight gain with 2.5% CG | UCG jelly (10% × 8 days) \((50)\) | | | | |
| Undegraded \( \delta \) | Rat | 0.25 | 2.5 | 2 days | AOM injection | | UCG solid gel (20 mg/kg ip) prior to CG | | | | |
| 2. Undegraded \( \kappa \), \( >100,000 \) | Rat | 0.5, 1.5, 5 | 2–3 days | AOM injection | 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 DGG, 8-fold with UCG. | | | | |
| Degraded \( \kappa \), \( 20,000 \) | Rat | 0.5, 1.5, 5 | \( \leq 91 \) days | AOM injection | | | | | | | |
| 3. Degraded \( \kappa \), \( 30,000 \) | Guinea pig | 2, 2.5, 5 | 7 days | AOM injection | Cecal ulcerations; foamy macrophages; small epithelial ulcerations at 2 days. | | | | | |
| 4. Degraded, \( 20,000–30,000 \) | Guinea pig | 3 | 5.8 | 2–3 days | Microscopic mucosal changes from cecum to rectum; apparent macro-molecule absorption by colonic epithelium, macrophage infiltration, macrophages with vacuoles. | | | | | |
| 5. Degraded \( \delta \) | Guinea pig | 1, 2, 3 | 2, 3, 4 | 2 weeks | Retarded cell growth caused cell death; at 0.25g/L inhibited DNA synthesis by 20%. | | | | | |
| 6. Degraded \( \delta \) | Rat ileal cell monolayers | 0–1.5 g/L | 19, 30, 54 hr | Media | | | | | | |
| 7. Undegraded \( \lambda \), \( 300,000 \) | Rat | 5 | 4 weeks | AOM injection | Increased permeability to (PH) PEG-900; ulcerations in guinea pig, crypt abscesses, macrophage infiltration. | | | | | |
| 8. Degraded \( \epsilon \) | Rat, Guinea pig | 5 | 5 | 4 months | | | | | | | |
| 9. Undegraded \( \kappa \) | Rat | 6 | 0.8 | 24 weeks | | | | | | | |
| 10. Degraded \( \lambda \) | Rabbit | 1 | 8 weeks, 12 months, 12 months, 28 months | AOM injection | Ulcerative lesions at 8 wks; at 12 months had chronic inflammatory changes. | | | | | |
| 11. Degraded \( \kappa \) | Mice | 10 | 10 days | AOM injection | 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. | | | | | |
| 12. Degraded, \( 20,000–40,000 \) | Cultured rat hepatocytes or intestinal mucosal cells | 1 mg/10 mL or 1 mg/100 mL | 20 hr | AOM injection | CG able to penetrate intestine; CG in mesenteric lymph node, and macrophages of villus and lamina propria. | | | | | |
| 13. Undegraded \( \kappa \), \( \lambda \), and \( \epsilon \) | Rat | 0.5 | 0.15–0.25 | 90 days | AOM injection | | CG in mesenteric lymph node, and macrophages of villus and lamina propria. | | | | |
| 14. Degraded \( \delta \) | Guinea pig | 5 | 5 days | AOM injection | | | | | | | |
| 15. Degraded \( \delta \) | Guinea pig | 5 | 14 days | AOM injection | | | | | | | |
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 Type | Animal | %CG (g/kg bw/day) | Duration | Route | Additional exposure | Digestive/ systemic | Histopathologic changes | Neoplastic changes | Reference |
|-----------------|--------|-------------------|----------|-------|---------------------|---------------------|------------------------|---------------------|-----------|
| 16. Degraded<sup>a</sup> | 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 (15% vs. 2.5%) and colon (10% vs. 4.5%) with CG than occurred with DMH alone. | | | (64) |
| 17. Degraded, (<20,000–40,000)<sup>a</sup> | 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 | Guinea pig | 3 | 3 weeks | Drink | Diarrhea after 7 days. Moribund after 9 days of CG. | No colonic lesions seen. | | | (66) |
| 19. Degraded (<20,000–40,000)<sup>a</sup> | 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<sup>c</sup> | Guinea pig | 5 | ≤ 28 days | Drink | Cecal lesions after 24 hr; confluent ulcerations after 7 days. Macrophage infiltration. | No difference in ulcerations from control. | Increased incidence of benign mammary tumors and testicular neoplasms (at 2.5% level) in rats only. | Squamous metaplasia of rectal mucosa at 2 weeks; extended after no longer being fed CG. | (68) |
| 21. Undegraded, (900,000), (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<sup>a</sup> | 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<sup>d</sup> | Guinea pig | 2 | 2 weeks | Drink | Loose stools by 2 weeks | 100% with colonic ulcerations; 75% had over 200 ulcers. | | | (34) |
| 24. Degraded<sup>d</sup> | Guinea pig | 5 | 21 days | Diet | 17/22 died by day 21 | All had mucosal ulcerations from cecum to rectum by day 14. | Squamous cell carcinomas, adenocarcinomas, adenomas. 32% and fed 10% diet had tumors. 100% incidence of metaplasia with 5% drink. | | (71) |
| 25. Degraded (<20,000–40,000)<sup>a</sup> | Rat | 10, 5, 1, 5, 1 or 5 | ≤ 24 months | 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. | | | (72) |
| 26. Undegraded<sup>c</sup> | 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. κ, (8,700–145,000)<sup>f</sup> | 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, (185,000–214,000) | Rat | 5 | 13 weeks | Diet | | | | | |
| κ, (5,000–145,000) | Guinea pig | 2 | 7–10 weeks | Diet | | | | | |
| κ, (5,000–145,000) | Guinea pig | 1 | 2–3 weeks | Drink | | | | | |
| κ, 8,500–275,000 | Guinea pig | 1 | 2–3 weeks | Drink | | | | | |
| λ, (5,000–145,000) | Guinea pig | 1 | 2–3 weeks | Drink | | | | | |
| Undegraded κ/λ, (185,000) | Rhesus monkey | 0.05, 0.2, 0.5 | Stomach tube | | | | | | |
| κ | 0.2 | Stomach tube | | | | | | |

Continued, next page
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 (69). In rats, the ulcerative lesions appeared initially in distal colon and rectum (48). Undegraded and degraded carrageenan have been associated

Table 3. Continued

| Experiment | Type of Effects |
|------------|----------------|
| 28. κ, (314,000) | Guinea pig | 1 | 2 weeks | Drink | Cecal ulceration not seen with κ or λ, ι fractions of MW 21,000–107,000 led to ulcers of cecum, crypt abscesses, and epithelial thinning. ι fractions absorbed and seen in vacuolated macrophages. Intense lysosomal enzymatic activity in macrophages of lamina propria. |
| 29. Degraded κ, (314,000), λ, (275,000), μ, (20,800), ι, (145,000), κ, (88,000), λ, (39,000), μ, (21,000), ι, (8,700), (5,000) | Rhesus monkey | 2 | 10 weeks | Drink | Macrophages given DCG had fibrillar material and vacuolations. |
| 30. Degraded κ, (314,000), λ, (275,000), μ, (20,800), ι, (145,000), κ, (88,000), λ, (39,000), μ, (21,000), ι, (8,700), (5,000) | Rhesus monkey | 1 | 10 weeks | Drink | Vacuolations seen with UCG. |
| 31. Degraded κ, (0.05, 0.2, 0.5) | Guinea pig, 0.25, 0.5 | ≤ 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 ulcers at 4 weeks. |
| 32. Degraded κ, (0.05, 0.2, 0.02) | Guinea pig, 2, 0.2, 0.02 | 12 months, 10 months, 3 months | Drink, In milk | Diarrhea, 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. |
| 33. Degraded κ, (0.05, 0.2, 0.5) | Guinea pig, 2, 0.2, 0.02 | 12 months, 10 months, 3 months | Drink, In milk | Diarrhea, Severe diarrhea in 3 days with 5% DCG contained within macrophages of spleen, liver, kidney, small and large intestine; cecal and colonic ulcers at 4 weeks. |
| 34. Undegraded κ, (0.05, 0.2, 0.5) | Guinea pig, 0.25, 0.5 | ≤ 4 weeks | Drink | Severe diarrhea in 3 days with 5% DCG contained within macrophages of spleen, liver, kidney, small and large intestine; cecal and colonic ulcers at 4 weeks. |
| 35. Degraded κ, (0.05, 0.2, 0.5) | Rhesus monkey, 0.5–2 | 7–14 weeks | Drink, 7–14 weeks, then recovery for 20–24 weeks for some before sacrificed | Diarrhea, hemorhage, ulcerations of colon; hypertrophy of mesenteric lymph nodes and granulomas; multiple crypt abscesses; dose effect present. |
| 36. Undegraded κ, (0.05, 0.2, 0.5) | Rhesus monkey, 0.5–2 | 7–14 weeks, then recovery for 20–24 weeks for some before sacrificed | Drink | Diarrhea, hemorhage, ulcerations of colon; hypertrophy of mesenteric lymph nodes and granulomas; multiple crypt abscesses; dose effect present. |
with epithelial cell loss and erosions in rats \(^{(31,65,70,87,93)}\). 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

| Type of carrageenan, molecular weight | Animal | %CG (g/kg bw/day) | Dose | 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. Patients had colon malignancy with colectomy planned to follow. CG exposure, no ulcerations seen. No lesions seen. No lesions seen. | | | | |
| Degraded\(^*\) | Guinea pig | 1, 2, 5 | 1–45 days | Drink | | | | | | | | |
| Degraded\(^*\) | Guinea pig | 2 | 1–45 days | Drink | | | | | | | | |
| Degraded | Humans | 5-g dose | 10 days | Diet | | | | | | | |
| Degraded | Ferret | 1.5 | 28 days | Tube | | | | | | | |
| Degraded | Squirrel monkey | 1.5 | 28 days | Tube | | | | | | | |
| Degraded | Rabbit, mouse | 1.5 | 28 days | Tube | | | | | | | |
| Undegraded | Rat | 5 | 56 days | Drink | Sl diarrhea | Diarrhea | | | | | |
| Undegraded | Rat | 5 | 6 months | Diet | | | | | | | |
| Undegraded | Hamster | 5 | 56 days | Diet | | | | | | | |
| 37. Degraded\(^*\) | Rat | 5 | 6–10 | 0.5–5.0 | 25 weeks | Drink | 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. | | | |
| Degraded\(^*\) (C16, C), (20,000–30,000)\(^{\text{III}a}e\) | 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. | | | | | |
| 38. Undegraded\(^{\text{III}a}e\) | Rhesus monkey | 1 | 7–12 weeks | Drink | | No changes in liver. | | | | | |
| Degraded\(^*\) (C16, C), (20,000–30,000)\(^{\text{III}a}e\) | Rhesus monkey | 0.5, 1, 2 | 7–12 weeks | Drink | | Membrane-bound vacuoles with fibrillar material in RE cells of liver. | | | | | |
| 39. Degraded\(^*\) | 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. | | | | | |
| 40. Degraded\(^{\text{III}a}e\) | Rat | 5 | 6 months | Drink | Ulceration of cecum 4/12, associated with stricture; marked glandular hyperplasia at ulcer margins. | | | | | | | |
| 41. Undegraded\(^*\) | Guinea pig | 5 | 2–4 weeks | Diet | Ulceration of mucosa as consequence of macrophage accumulation in lamina propria, then submucosa. | | | | | | | |
| Degraded\(^*\) | Guinea pig | 1 | 2–4 weeks | Drink | | | | | | | | |
| 42. Degraded\(^{\text{III}a}e\) | 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%, 80% of those fed 0.1%. | Hyperplastic mucosal changes, polypoidal lesions. | | | |
| 43. Degraded\(^*\) | Guinea pig | 4–5 | Drink | Mucosal erosions in cecum, rarely into colon in guinea pig; without erosion in rat or mouse. | | | | | | | |
| Degraded\(^*\) | Rat, mouse | ≥ 16.5, 0.07–4 | 28 days–6 months | Drink | | Multiple ulcerations of cecum; 80% had ulcerations. Crypt abscesses present; macrophages, with metachromatic material. | | | | | |
| 44. Undegraded | Guinea pig | 1 | ≤ 1.5 | 20–30 days | Drink | FOB+ | 100% had ulcerations; ulceration extended into distal colon and rectum. | | | | | |
| Degraded | Guinea pig | 5 | ≤ 2 | 20–30 days | Drink | Diarrhea by 10 days, FOB+ | Hemorrhagic and ulcerative lesions in cecum, colon, or rectum in all four species; crypt abscesses present. | | | | | |
| 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 mucus in stool. | | | | | |

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

\(^*\)Studies are associated with neoplastic changes, unlike studies predominantly demonstrating intestinal ulcerations.  
\(^\text{III}\)Increased proliferation or neoplasm and carrageenan alone.  
\(^\text{IV}\)Ulceraions and carrageenan alone.  
\(^\text{V}\)Neoplasms in which carrageenan promoted carcinogenesis.  
\(^\text{VI}\)Studies with uptake to lymph node or other site.  
\(^\text{VII}\)Study demonstrating breakdown to lower molecular weight.  
\(^\text{VIII}\)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. (88) 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 t-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/cm² over the mucosal surface of the cecum in the guinea pig.

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 in 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.
infiltration (35, 56, 63, 65, 68, 75, 76, 78–81, 83, 84, 88, 92, 102–104). Fibular material and metachromatic staining of the macrophages were observed. Notably, the macrophage lysosomes appeared to take up the fibular material and to become distorted and vacuolated. It appeared that colonic ulcers 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 fibular 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. 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 (39).

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 sulfatas and sulfate reductases can produce hydrogen sulfide gas or HS− from carrageenan. Carrageenan, as well as other sulfated polysaccharides, has been shown to stimulate H2S 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 k-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 ulcers, Delahunt et al. (56) observed an increased permeability to large molecules, such as [3H]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 vitro (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 negligibly 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 sulfateases, 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 ulcera
tions 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 underdegummed carrageenan that 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, 1997:251–311. 3. Schatzkin A. Available: http://rex.nci.nih.gov/ 4. Marcus R, Watt J. Danger of carrageenan in foods and their processing. In: Marine Colloids Division, filing of Food Additive Petitions; Hercules, Inc.; Notice of Receipt of Citizen Petition; request for Comments. Fed Reg 44:4943–4965. (1979). 5. National Research Council. Food Chemical Codex. 4th ed. Washington, DC:National Academy of Science, 1996. 6. Kitsukawa Y, Saito H, Suzuki Y, Kasanuki J, Tamura Y, Yoshida S. Effect of ingestion of icescapesan acid ethyl ester on carcino genesis in guinea pigs. Gastroenterology 102:1859–1866 (1992).

7. Marcus AJ, Marcus SN, Marcus R, Watt J. Rapid progression of ulcerative disease of the colon in newly-weaned guinea pigs by degraded carrageenan. J Pharm Pharmacol 41:423–426 (1989).

8. Ling K-Y, Bhalla D, Hollander D. Mechanisms of car rageenan injury of IEC8 small intestinal epithelial cell monolayers. Gastroenterology 95:1487–1495 (1988).

9. Calvert RJ, Reicks M. Alterations in colonic thymidine kinase enzyme activity induced by consumption of various dietary fibers. Proc Soc Exp Biol Med 205:45–51 (1994).

10. Delahunty T, DeJong H, Hollander D. Intestinal permeability changes in rodents: a possible mechanism for degraded carrageenan-induced colitis. Food Chem Toxicol 34:1135–1141 (1996).

11. Arakawa S, Okumura M, Yamada S, Ito M, Téjima S. Enhancing effect of carrageenan on the induction of rat colonic tumours by 1,2-dimethylhydrazine and its relation to jejuno-glucuronidase activities in feces and other tissues. J Nutr Sci Vitam (Tokyo) 32:481–485 (1986).

12. Kitano A, Masutomo T, Hiki M, Hashimura H, Yoshiyasu K, Okaiva K, Kuwajima S, Kobayashi K, Epithelial dyspla sia of the rabbit colon induced by degraded carrageenan. Cancer Res 46:1374–1379 (1986).

13. Fath RB, Descher EE, Winawer SJ, Dworkin BM. Degraded carrageenan-induced colitis in CF, mice. Gastroenterology 100:159–166 (1991).

14. Mori H, Ohbayashi F, Hirono I, Shimada T, Williams GM. Absence of genotoxicity of the carboxylic-sulfated polysaccharide carrageenan and dermatane in mammalian DNA repair and bacterial mutagenicity assays. Nut Cancer 6:92–97 (1984).

15. Capponi S, Miller K. Oral effect of administered food-grade carrageenan on antibody-mediated and cell-mediated immunity in the inbred rat. Food Chem Toxicol 22:615–621 (1984).

16. Jensen BH, Andersen JO, Poulsen SS, Olsen PS, Rasmussen SN, Hansen SH, Hvidberg DF. The prophylactic effect of f-aminomethylhistidine on 1,2-dimethylhydrazine diethyl ester on carrageenan-induced colitis in guinea pigs. Scand J Gastroenterol 10:299–303 (1984).

17. Olsen PS, Kirkegaard P, Poulsen SS. The effect of ileo-transversostomy on carrageenan-induced colitis in guinea pigs. Scand J Gastroenterol 18:407–413 (1983).

18. Kawamura A, Shibata M, Togei K, Otsuka H. Effect of dietary degraded carrageenan on intestinal carcino genesis in rats treated with 1,2-dimethylhydrazine dihydrochlo ride. Tokushima J Exp Med 29:125–129 (1982).

19. Hirono I, Sumi Y, Kuhara K, Miyakawa M. Effect of degraded carrageenan on the intestine in germline rats. Toxicol Lett 6:207–212 (1981).

20. Norris AA, Lewis AJ, Zettler LJ. Inability of degraded car rageenan fractions to induce inflammatory bowel ulceration in the guinea pig. J Pharm Pharmacol 33:612–613 (1981).

21. Ohsahi Y, Ishioka TT, Wakahayashi K, Kuwabara N. A study of carcinogenicity induced by degraded carrageenan arising from squamous metaplasia of the rat colon. Cancer Lett 67:189–194 (1992).

22. Ohsahi Y, 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).

23. Rustia M, Shubik P, Patil K. Lifespan carcinogenicity tests with native carrageenan in rats and hamsters. Cancer Lett 11:1–10 (1981).

24. Ohsahi Y, Kitamura S, Wakahayashi K, Kuwabara N, Fukuda Y. Irreversibility of degraded carrageenan-induced colorectal squamous metaplasia in rats. Gann 70:391–392 (1979).
