Acta path. microbiol. scand. Sect. A, *88*: 135–141, 1980.

STEREOMICROSCOPIC AND HISTOLOGIC CHANGES IN THE COLON OF GUINEA PIGS FED DEGRADED CARRAGEENAN

P. SKOV OLSEN and S. SEIER POULSEN

Anatomy Department B, University of Copenhagen, Denmark

Olsen Skov, P. & Poulsen Seier, S. Stereomicroscopic and histologic changes in the colon of guinea pigs fed degraded carrageenan. Acta path. microbiol scand. Sect. A, *88*: 135–141, 1980.

A colitis-like state was induced in Guinea Pigs fed degraded carrageenan orally. By means of a combined semimacroscopic and histologic technique the course of the disease was followed during 28 days. The changes were primarily seen and became most prominent in the caecum. The first lesions were observed following 24 hours of treatment as small rounded foci initially with degenerative changes and inflammation in the surface epithelium, later forming superficial focal ulcerations. Ulcerative changes gradually progressed during the experiment, forming linear and later large, geographical ulcerations. Topographically the ulcerative process was strongly related to the larger submucosal vessels. Nonulcerated parts of the mucosa were not changed until following 7–14 days of treatment. The mucosa became bulging, granulated and finally villus-like. Accumulation of macrophages was found under the surface epithelium after 7–14 days. Possible pathogenetic mechanisms are discussed, especially the development of the early lesions and the significance of the macrophages.

Key words: Colon; carrageenan; stereomicroscopy; histology.

Steen Seier Poulsen, Anatomy Department B, University of Copenhagen, the Panum Institute, Blegdamsvej 3, DK-2200 Copenhagen N.

Received 13.vi.79 Accepted 2.xi.79

Carrageenan is a sulphated polysaccharide derived from the red seaweed, Euchema Spinosum. It is widely used in the food industry as a stabilizer and emulgator (5)

In 1969 *Marcus and Watt* (11, 20) reported that a colitis-like disease can be induced in laboratory animals by oral administration of degraded carrageenan in the drinking water. Undegraded (native) carrageenan was found to have the same effect but was less effective than the degraded form (20).

The colitis-inducing effect of degraded carrageenan has been confirmed by other authors (3, 8, 12, 16, 19). Guinea pigs and rabbits are the most susceptible species (11, 22, 23) but colonic changes have been provoked also in rats and mice (11, 23) and in rhesus monkeys (3)

Several reports (2, 3, 8, 12, 19, 20, 22) have dealt with the histopathology of the colonic lesions induced by degraded carrageenan, but the results have been varying and in some cases conflicting, both as regards the development of the lesions and their localization. *Watt and Marcus* (22) found the changes most pronounced in the caecum, but also severe in the rectum and moderate in the rest of the colon. *Sharratt et al.* (16, 17) found the disease almost confined to the caecum, whereas *van der Waaij et al.* (19) observed the most severe changes in the distal colon. In the same way some reports (2, 8, 16) have considered the well-documented macrophage cytotoxic effect of carrageenan (4) to be the most important factor in the pathogenesis of the ulcerations, while other reports (12, 19) hardly mention the macrophages.

The histopathological technique of investigating gastrointestinal specimens is improved and facilitated by the application of surface staining (9, 13, 14, 15). Following surface staining the structure of the mucosal surface is visualized in the stereomicroscope. Changed areas can be localized and subsequently examined histologically.

The aim of the present study has been, by means of the combined stereomicroscopic and histologic technique, to investigate the development of the colonic changes induced by degraded carrageenan and to describe the early lesions and the localization of the disease related to the duration of the treatment.

MATERIAL AND METHODS

42 smooth-haired Guinea pigs, average weight 560 g, were given degraded carrageenan 5% in the drinking water. They were sacrified in groups of six after 1, 2, 4, 7, 14, 21 and 28 days. During the experiment the animals received Purina Lab Chow, *ad libitum*. The control material consisted of six untreated Guinea pigs.

For preparation of the colonic specimens the Guinea pigs were anaesthetized with Nembutal 50g/100g i.p. The abdomen was opened and after ligation at the distal end of ileum and rectum the entire colon was fixed *in situ* by direct intraluminal injection of 10% formalin. The colon was then removed and placed in formalin. After fixation for 10 minutes it was cut open antimesenterically and suspended on a polyethylene plate with fine needles. After further fixation for 24 hours the specimens were washed in water and stained with Alcian green 3BX, 0.5% in 1% acetic acid for five minutes (9).

The entire specimens were studied under the stereomicroscope. Appropriate areas were photographed and subsequently taken out for histological examination. The section lines were indicated on stereomicroscopic photographs to permit an exact correlation between the stereomicroscopic and the histologic findings. The histological specimens were stained with PAS-Hematoxylin-Aurentia and also with Alcian blue 1% in 0.1n HCl (pH 1) for identification of macrophages containing carrageenan (7).

RESULTS

In the surface-stained specimens the crypt openings and the goblet cells clearly stand out dark green on a bright green background. In the controls the pattern of crypt openings was regular (Fig. 1). The goblet cells were mainly concentrated in and just around the crypt openings and were only occasionally seen in the surface epithelium. The mucosal surface was even.

Early Lesions

In the caecum and ascending colon, delicate changes were observed already after 24 hours of treatment. In the stereomicroscope they were seen as small rounded, dark foci 0.5–1 mm in diameter (Fig. 2a). The luminal parts of the crypt tubules were seen in the areas as white rings, while the interjacent surface was stained dark green by the Alcian. In the caecum the small foci were regularly arranged in longitudinal rows closely related to the course of the larger submucosal vessels overlying the three tenia coli. In the ascending colon they were observed along the attachment of the mesentary.

Histological examination of the small foci (Fig. 2b) primarily showed a pronounced flattening of the surface epithelium with disorganization of the epithelial cells, lack of nuclear polarity, and infiltration with a few leucocytes. In areas with changes a little more advanced, the crypt epithelium was also affected. The crypt tubules seemed to degenerate, being narrow and shortend, leaving a gap between the base of the crypt and the muscularis mucosae. In some areas the crypt tubules had disappeared completely. In the lamina propria a moderate infiltration with leucocytes was observed.

Ulcerative Changes

After 2 and 4 days epithelial defects were seen in the small foci, forming punctuate ulcerations with well-defined edges (Fig. 3). The number of foci had increased and they had extended also along the transverse ramifications of the longitudinal vessels (Fig. 4).

After 7 days many of these small, punctuate erosions had become confluent, forming shallow, linear ulcerations (Fig. 4) mainly oriented longitudinally yet following the course of the larger vascular structures.

The ulcerative changes gradually progressed and in the fully developed stages as seen after 21 and 28 days of treatment, the ulcerations were large, irregular and confluent, involving large parts of the mucosal surface (Fig. 5).

Histologically all the ulcerations were superficial. Penetration of the lamina muscularis mucosae was not observed. In the bottom of the ulcerations acute inflammatory exudate and granulation tissue was found.

The Appearance of the Non-Ulcerated Mucosa.

Within the first week of treatment the mucosal surface was flat as in the controls. After 7 days a local bulging was observed in the mucosa surrounding the ulcerations. The remaining surface was normal.

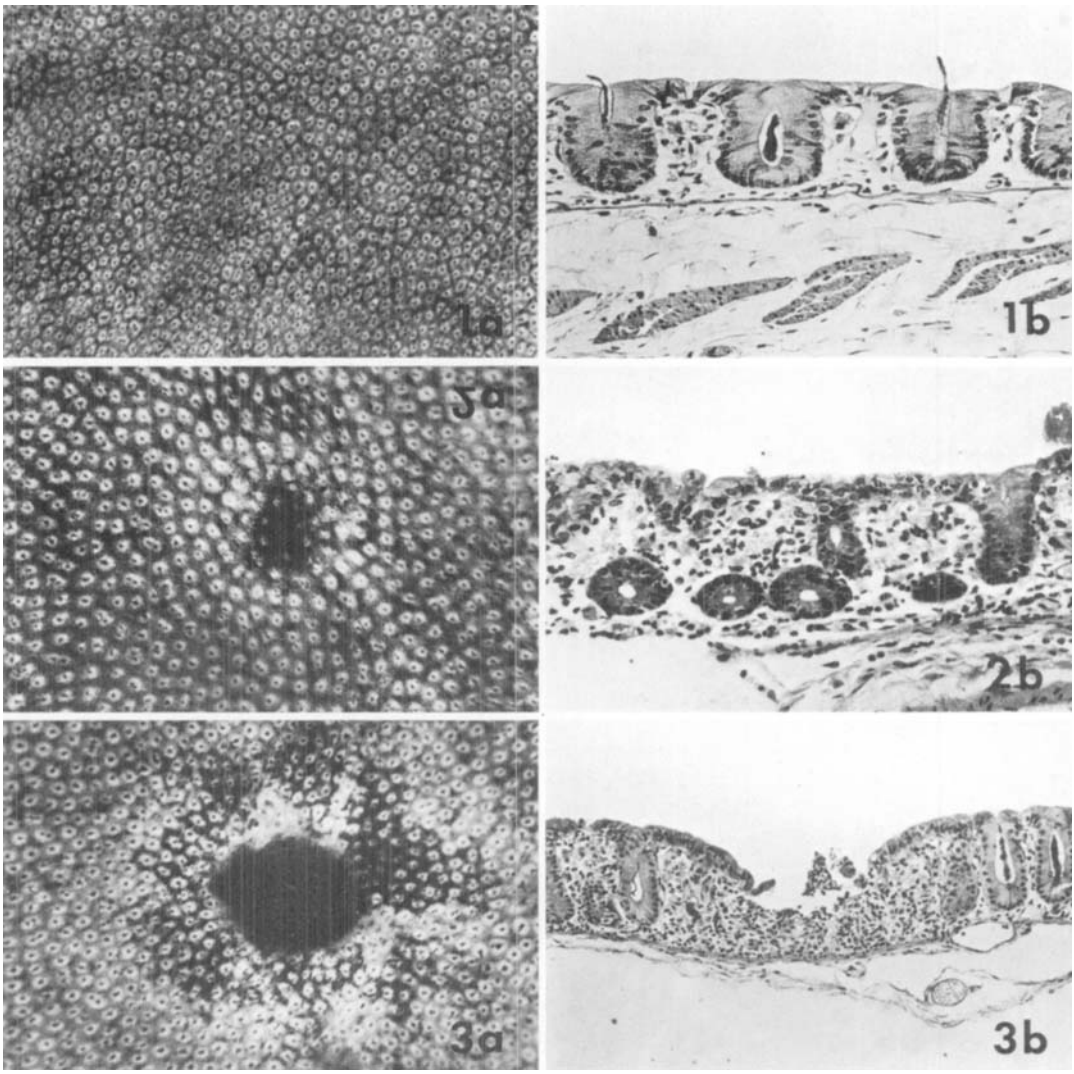


Fig. 1a. Stereomicroscopic appearance of the normal Guinea pig colon. The crypt tubules are seen as white rings with the dark crypt opening in the center. (Alcian × 20).

Fig. 1b. Corresponding histological section showing the appearance of the normal mucosa. (PAS-Aurentia × 200).

Fig. 2a. An early focal lesion observed as a rounded heavily stained area with blurred outlines of crypt openings. (Alcian × 20).

Fig. 2b. Corresponding histological section showing affection of the surface and crypt epithelium. The epithelium is infiltrated by inflammatory cells, and the epithelial cells are disarranged and flattened. (PAS-Aurentia × 200).

Fig. 3a. Punctuate ulceration. A well defined, superficial lesion surrounded by a mucosa with increased number of goblet cells in the surface epithelium. (Alcian × 20).

Fig. 3b. Corresponding histological section showing superficial ulceration. Lamina muscularis mucosa is unaffected. (PAS-Aurentia × 200).

Diffuse changes in the mucosal surface structure were observed in the caecum and ascending colon after 14 and 21 days. They were most pronounced in the caecum where the entire surface was bulging and granulated. After 28 days the bulging had progressed, giving the surface a villus-like appea-

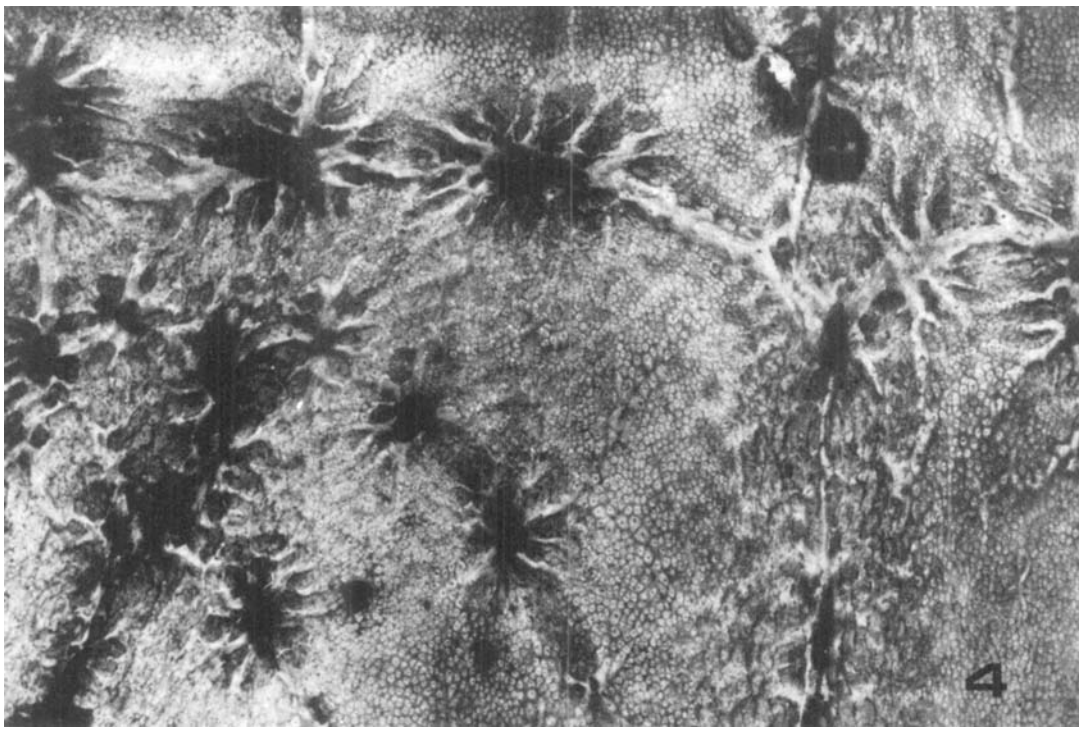


Fig. 4. Several punctuate and linear ulcerations following the course of the submucosal vascular structures. The mucosa surrounding the ulcerations is granulated. (Alcian × 6, 3).

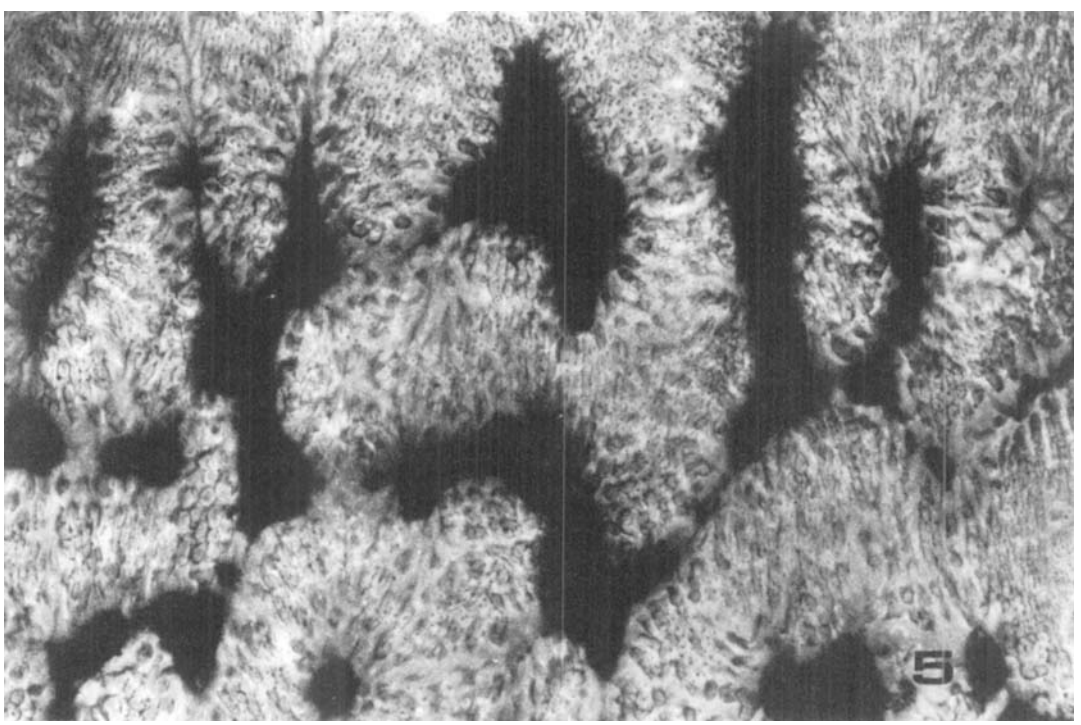
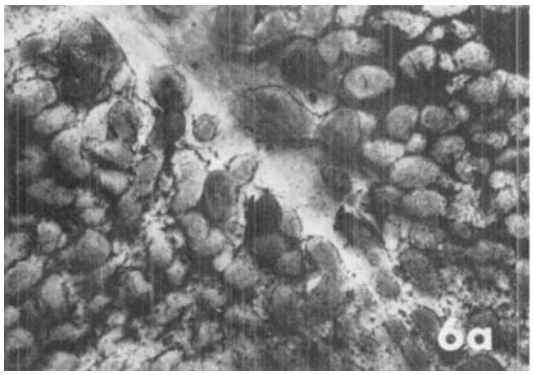


Fig. 5. Several large ulcerations surrounded by a villus-like mucosa. (Alcian × 6, 3).



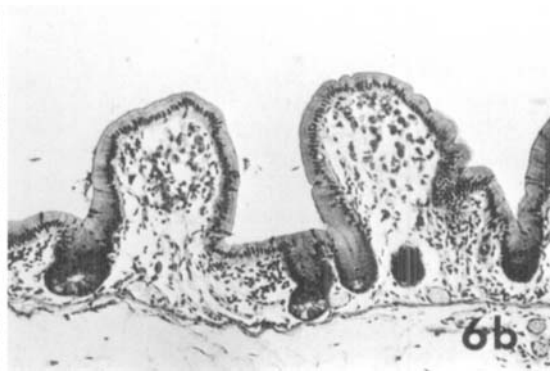


Fig. 6a. Villus-like mucosa. Crypt openings may be seen between the villus-like structures. Plenty of goblet cells are seen in the surface epithelium. (Alcian × 10).

Fig. 6b. Corresponding histological section showing villus-like structures. Large numbers of PAS-positive macrophages are accumulated in the stroma beneath the surface epithelium. (PAS-Aurentia × 200).

rance (Fig. 6a). In the ascending colon the changes were less pronounced. The surface was swollen, slightly granulated, often of a gyriform appearance. In the distal colon, changes were observed only around the ulcerations.

Histologically, the stroma in non-ulcerated areas was normal within the first week. In the bulging and villus-like mucosa observed in the later stages, plenty of large PAS-positive macrophages were observed, mostly located just beneath the surface epithelium (Fig. 6b). The accumulation of macrophages was evident after 7–14 days of treatment. Besides, there was edema and a moderate infiltration with acute and chronic inflammatory cells. The space between the crypt tubules was increased, but the epithelial cells in the crypt and surface epithelium appeared normal.

Localization of Disease

Changes can be classified as focal (ulcerative) and diffuse (surface contours, macrophage infiltration and edema). Focal changes developed considerably earlier than the diffuse changes and in less-affected areas they were still the only changes.

The caecum was always the most affected part of the colon. Small, punctuate lesions were observed in the caecum already after 24 hours, quickly progressing to form superficial erosions. The ulcerative changes gradually progressed during the 4 weeks of treatment from puncutate to linear and later large, confluent ulcerations. Diffuse changes in non-ulcerated parts of the mucosa were not observed until 14 days after initiation of treatment.

In the rest of the colon all changes appeared later and they never became as advanced as in the caecum. Apart from this they had the same morphology. Punctuate ulcerations were seen in the proximal colon after 4 days. They gradually spread distally and were found in the distal colon and rectum after 21 days. In these parts of the colon they remained small, never exceeding 2–3 mm in diameter. Changes in non-ulcerated areas were only moderately developed and were not observed in the distal colon. The disease involves the proximal colon in the first place and subsequently spreads distally in continuity to the distal colon.

In the rectum, however, diffuse mucosal inflammation, increased space between the crypt tubules, and degeneration of the epithelium might be observed in the later stages.

DISCUSSION

When investigating the development of the colonic changes induced by degraded carrageenan it is essential to define the early lesions. Applying similar doses of carrageenan, the early lesions previously have been described after 5–7 days (2, 8, 12). A sequence of events preceding ulceration has been proposed (2):

1) Penetration of carrageenan into the lamina propria.
2) Accumulation of macrophages containing carrageenan under the surface epithelium.
3) Release of lysosomal enzymes from the macrophages inducing inflammation and edema in the stroma followed by
4) degenerative changes and inflammation in the surface epithelium finally leading to
5) ulceration.

In the present investigation early lesions were identified already after 24 hours. The first changes observed were:

1) Focal affection of the surface epithelium.
2) Affection of the crypt epithelium and acute inflammation in the mucosal stroma, and finally
3) focal ulceration.

Accumulation of macrophages was not observed at this early stage. A macrophage reaction was not evident until 7–14 days after initiation of treatment and was mostly observed in the bulging or villus-like mucosa. Considering that lesions developed within the first 24 hours of treatment, the macrophage accumulation is hardly a part of the primary ulcerative process but rather a secondary foreign body reaction. This is, moreover, supported by the fact that even following direct s.c. injection of carrageenan, accumulation of macrophages is only demonstrated after 5 days (24). Carrageenan is cytotoxic to macrophages (4). It is taken up and stored in the macrophages which are unable to sequester the compound (2, 10). Accumulation of macrophages and release of lysosomal enzymes may be involved in the development of the generalized – non ulcerative changes – as the heavy bulging of the mucosa as seen in the fully developed stages. This is in agreement with the observation in other studies that the aggregations of macrophages are connected with the bulging and villus-like mucosa (2).

It has been discussed how the large carrageenan molecules (MW 30.000) are able to penetrate the intact surface epithelium (2, 8), which is nescessary if the macrophage reaction is initiating the ulcerative process. The explanation may be that the permeability of the epithelium is increased secondarily to the acute affection of the surface epithelium.

This may also be the reason why the focal early changes are detected in the stereomicroscope as areas with increased stainability. The large Alcian molecules are probably able to penetrate the damaged epithelium in the focal areas and are retained in the lamina propria, staining these areas dark green contrasting to the rest of the mucosal surface where the goblet cells are stained selectively.

In the present study strong topographical relationships have been observed between the larger submucosal vascular structures and the initial ulcerative process. This relationship has not been noticed in previous studies, and we have no explanation for it. Further investigation will be needed to evaluate its pathogenetic significans.

The reason why the disease is most pronounced in the caecum of the Guinea pig might be that Guinea pigs, like other ruminants, have a large caecum in which the intestinal contents have a long transit time (18). The mucosal surface in the caecum therefore is exposed earlier and for a longer time to the carrageenan than is the rest of the colon. Moreover, some of the carrageenan may be absorbed or decomposed in the caecum, resulting in reduced contents of carrageenan in the rest of the colon.

It has been well documented that carrageenan is a biologically very active compound (6) with several possible side-effects. The important question is only whether it is absorbed or not. It has been discussed whether carrageenan might be an etiological factor in human ulcerative colitis (8, 11, 16, 17, 21, 22). This has been rejected since there are several pathological differences between experimental and human ulcerative colitis (8, 16, 17). Moreover, only Guinea pigs and rabbits are sensitive to the undegraded form of carrageenan, the large molecules of which do not seem to be absorbed, for instance, by rats and monkeys (3, 8, 10). On the other hand, it is possible that carrageenan is harmful to patients who already suffer from ulcerative colitis. In the present paper it has been proposed that carrageenan penetrates the surface epithelium only when this has been damaged primarily. In ulcerative colitis, atrophy and necrosis are frequently observed in the surface epithelium. This may increase the risk of penetration of carrageenan. If the widespread application of carrageenan in the food industry should be questioned at all, it would be reasonable initially to concentrate on this group of patients in order to find out whether carrageenan is absorbed by them or not, and if it is, whether it has any harmful effects.

REFERENCES

1. *Abraham, R., Goldberg, L. & Coulston, F.:* Uptake and storage of degraded carrageenan in lysosomes of reticuloendothelial cells of the rhesus monkey, Macaca Mulattae. Exp. Mol. Pathol. *17:* 77–93, 1972.
2. *Abraham, R., Fabian, R. J., Goldberg, M. B. & Coulston, F.:* Role of lysosomes in carrageenan-induced cecal ulceration. Gastroenterology. *67:* 1169–1181, 1974.
3. *Benitz, N. F., Goldberg, L. & Coulston, F.:* Intestinal effects of carrageenan in the rhesus monkey, Macaca Mulattae. Fd. Cosmet. Toxicol. vol. *11*, Pergamon Press. pp. 565–575, 1973.
4. *Catanzaro, P. J., Schwartz, H. J. & Graham, R. C.:* Spectrum and possible mechanism of carrageenan cytotoxicity. Amer. J. Path. *64:* 387–404, 1971.
5. *Christensen, O.:* Carrageenan, a useful food additive. Manuf. *39:* 49, 1964.
6. *Di Rosa, M.:* Biological properties of carrageenan review. J. Pharm. Pharmacol. *24:* 89–102, 1972.

7. *Gangolli, S. D., Wright, M. G. & Grasso, P.:* Identification of carrageenan in mammalian tissues: An analytical and histochemical study. Histochemical Journal. *5:* 37–48, 1973.
8. *Grasso, P., Sharrat, M., Carpanini, F. M. B. & Gongolli, S. D.:* Studies on carrageenan and large bowel ulceration in mammals. Fd. Cosmet. Toxicol., vol. *11*, Pergamon Press. pp. 555–561, 1973.
9. *Landboe-Christensen, E. & Parapat, S.:* Staining of the mucosal surface of the human digestive tract. IRCS, *2*, 1034, 1974.
10. *Mankes, R. & Abraham, R.:* Lysosomal dysfunction in colonic submucosal macrophags of rhesus monkeys caused by degraded Iota carrageenan. Proc. Soc. Exp. Biol. Med. *150:* 166–170, 1975.
11. *Marcus, R. & Watt, J.:* Seaweeds and ulcerative colitis in laboratory animals. Lancet. *2:* 489–490, 1969.
12. *Onderdonk, A. B., Hermos, J. A., Dzink, J. L. & Bartlett, J. G.:* Protective effect of Metronidazole in experimental ulcerative colitis. Gastroenterology. *74:* 521–526, 1978.
13. *Poulsen, S. S. & Szabo, S.:* Mucosal surface morphology and histologic changes in the duodenum of the rat following administration of cysteamine. Br. J. exp. Path. *58:* 1–8, 1977.
14. *Poulsen, S. S. & Petri, M.:* Surface staining of small intestinal biopsies. Scand. J. Gastroenterol. *12:* 235–240, 1977.
15. *Poulsen, S. S., Christensen, N. C., Petri, M & Jarnum, S.:* Stereomicroscopic examination of stained rectal biopsies. Scand. J. Gastroenterol. *13:* 605–608, 1978.
16. *Sharrat, M., Grasso, P., Carpanini, F. M. B. & Gangolli, S. D.:* Carrageenan ulceration as a model for human ulcerative colitis. Lancet. *2:* 932, 1970.
17. *Sharrat, M., Grasso, P., Carpanini, F. M. B. & Gangolli, S. D.:* Carrageenan ulceration as a model for human ulcerative colitis. Lancet. *1:* 192–193, 1971.
18. *Varga, F.:* Transit time changes with age in the gastrointestinal tract of the rat. Digestion. *14:* 319–324, 1976.
19. *Waaij, D. van der, Cohen, B. J. & Anver, D. V. M.:* Mitigation of experimental inflammatory bowel disease in guinea pigs by selective elimination of the aerobic gram-negative intestinal microflora. Gastroenterology. *67:* 460–472, 1974.
20. *Watt, J. & Marcus, R.:* Ulcerative colitis in the guinea pig caused by seaweed extract. J. Pharm. Pharmacol., *21*, suppl., 1875–1885, 1969.
21. *Watt, J. & Marcus, R.:* Hyperplastic mucosal changes in the rabbit colon produced by degraded carrageenan. Gastroenterology. *59:* 760–768, 1970.
22. *Watt, J. & Marcus, R.:* Carrageenan-induced ulceration of the large intestine in the guinea pig. Gut. *12:* 164–171, 1971.
23. *Watt, J. & Marcus, R.:* Experimental ulcerative disease of the colon in animals. Gut. *14:* 506–510, 1973.
24. *Williams, G.:* Histological study of the connective tissue reaction to carrageenan. J. Path. Bact. *73:* 557–563, 1957.