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Carrageenan-induced ulceration of the large intestine in the guinea pig

J. WATT AND R. MARCUS

From the Department of Pathology, University of Liverpool, and the Surgical Unit, Clatterbridge Hospital, Bebington, Cheshire

SUMMARY A 5% aqueous solution of degraded carrageenan derived from the red seaweed *Eucheuma spinosum* was fed to guinea pigs in their drinking water over a period of 20-45 days. Occult blood in the faeces and multiple ulcers in the caecum, colon and rectum occurred in 100% of animals by the 30th day. The clinical and pathological features bear a close resemblance to human ulcerative colitis.

The method provides a simple experimental model for the study of various aspects of the pathology of ulcerative lesions in the large intestine as well as the effects of therapeutic agents.

Experimental 'colitis' in animals has been produced by a variety of methods including the injection of cholinergic and adrenergic drugs, prolonged administration of histamine and histamine-releasers, local injection of collagenase, oral or intra-arterial administration of lysozyme, as well as several immunological procedures. The subject has been reviewed by Kirsner (1961) who considered that none of these experimental reactions resembles human ulcerative colitis and that their significance in relation to the clinical condition remains in doubt. More recently, haemorrhagic ulcerative 'colitis' has been produced in rats by immunization with living *E. coli* injected subcutaneously in Freund's adjuvant. The symptoms, course, and histological characteristics of the lesions are reported as being very similar to human ulcerative colitis (Zweibaum, Morard, and Halpern, 1968).

In this paper we describe a simple method for the production of ulcers in the large intestine of the guinea pig requiring only the addition of a degraded carrageenan to the drinking water. The method is one which may be used as an experimental model for the study of various aspects of the pathology of ulcerative lesions in this part of the alimentary tract, as well as the effects of therapeutic agents. It is based on observations made during investigations of the biological activity of degraded and undegraded carrageenans derived from a variety of seaweeds (Marcus and Watt, 1969).

Material and Method

Adult male albino guinea pigs (average body weight 620 g) were housed in separate cages and fed a standard cube diet (SG1) supplemented daily with fresh cabbage. Degraded carrageenan was obtained from a commercial source (Laboratoires-Glaxo, Paris), the carrageenan having been derived from the red seaweed *Eucheuma spinosum* and degraded by mild acid hydrolysis so as to retain about 29% sulphate content.

The degraded carrageenan was added to the drinking water to a concentration of 5% and was freshly prepared each day. It was supplied to the animals *ad lib* in inverted graduated measuring bottles fitted with a glass tube. Some spillage invariably occurred from the end of the tube during the time of drinking or immediately after. For this reason an accurate assessment of the daily fluid intake was not possible, although the total fluid supplied and the quantity remaining were charted each day. At a maximum, the daily intake of degraded carrageenan per animal was no greater than 2 g/kg body weight.

Fourteen guinea pigs received degraded carrageenan over periods from 20 to 45 days. Four animals in a control group received water without added carrageenan. Each day throughout the experiment the animals were weighed, the stools examined for occult blood using Hematest tablets (Ames Company), and both the faeces and urine tested for metachromasia with toluidine blue (Watt and Marcus, 1965).

The animals were killed using ether anaesthesia. The gastrointestinal tract was removed. The large bowel was emptied of faeces and distended with 10% formol saline. After fixation the intact colon was examined by transmitted light and the opened specimens both by transmitted and direct lighting.

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Tissues taken for histology included various parts of the large bowel, as well as portions of liver, kidney, mesenteric lymph nodes, and spleen fixed in 10% formol saline or absolute methyl alcohol. Sections were stained with haematoxylin and eosin, phloxine tartrazine, and toluidine blue.

Results

All animals receiving degraded carrageenan in their drinking fluid showed loss of weight which became apparent after the second week. The weight loss at the end of the experiment ranged from 80 to 150 g representing approximately 15 to 25% of the initial body weight.

By the end of the first week, most of the animals in the experimental group showed looseness of the stools. Tests for occult blood in the faeces were negative during the first two weeks. Thereafter, the tests became positive in over half the animals by the 18th day and in all animals in the experimental group by the 30th day. One of the animals which received degraded carrageenan for 45 days showed visible blood in the faeces.

Examination of both faecal smears and urine revealed positive metachromasia with toluidine blue as from the second day of the experiment in all animals receiving degraded carrageenan.

The control group of animals showed no loss of weight, no diarrhoea, no occult blood in the faeces, and negative metachromasia with toluidine blue both in faecal smears and urine throughout the entire experimental period.

INCIDENCE AND SITE OF ULCERATION IN THE LARGE INTESTINE

Ulcerative lesions in the large intestine occurred in 12 of the 14 animals in the experimental group receiving degraded carrageenan for 20 to 45 days. Four animals were killed between the 20th and 25th days; two showed ulceration of the caecum and proximal colon, and two no abnormality of the large intestine. Six animals killed on the 30th day and four between the 35th and 45th days all showed ulcerative lesions in various parts of the large intestine. Thus, in animals fed degraded carrageenan for 30 days or longer the incidence of ulceration in the large intestine was 100%.

The ulcers varied in severity. In general the extent and severity of the ulceration increased with the length of the experiment. From 20 to 25 days lesions were mainly in the caecum; from 30 to 45 days, the caecum, colon, and rectum were involved. The Table shows the distribution of ulceration in the group of six animals which had received degraded carrageenan for 30 days. Ulceration was present in the caecum in all six of these animals. The lesions in the colon were less extensive than in the caecum. In the rectum the lesions were severe and distributed diffusely over segments of bowel ranging from 20-40 cm in length.

The control group of animals killed at the end of the experiment showed no abnormality of the gastrointestinal tract.

Guinea Pig	*Area of Caecum Involved*	*Length of Ulcerated Segment (cm)*	
		Colon	*Rectum*
177	Proximal two-thirds	8	40
178	Proximal one-third	10	33
179	All parts	5	35
181	All parts	3	31
182	All parts	12	20
183	Proximal half	4	30

Table *Distribution of ulcerative lesions in the large bowel of guinea pigs fed degraded carrageenan for 30 days*

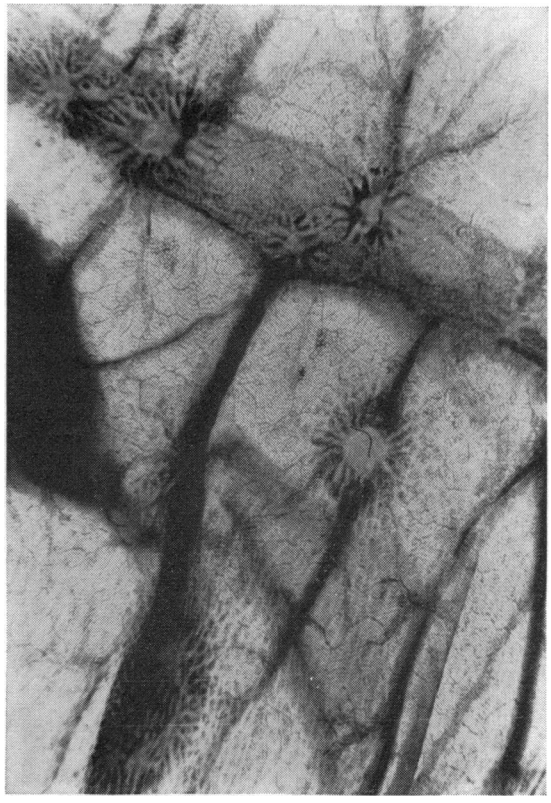

Fig. 1 *Guinea pig fed degraded carrageenan for 30 days. Multiple ulcers in caecum as viewed by transmitted light* ×7.

MACROSCOPIC FEATURES

In four guinea pigs which had received degraded carrageenan for 30 days or longer, multiple small rounded opacities (about 1 mm diameter) were visible through the serosa of the caecum. Otherwise no abnormality was noted on external examination of the large bowel in most of the animals in the experimental group.

The ulcerative lesions in the caecum were best seen by the use of transmitted light. They involved all parts of the caecum and were invariably multiple; in severe cases, hundreds of lesions were present. The ulcers were generally pin-head in size and rounded, oval or slightly irregular in outline (Fig. 1). Some lesions had coalesced to form larger ulcerated areas which were sometimes linear in shape (Fig. 2). Marginal congestion was always present. Ulcerative lesions in the colon were more difficult to see by transmitted light. On direct examination of the opened specimen, their presence was indicated by

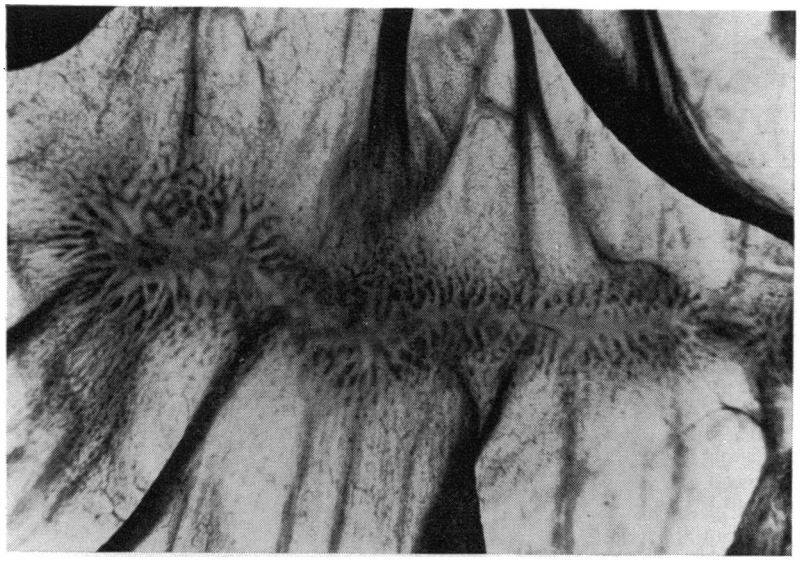

Fig. 2 *Linear ulceration of the caecum* $\times 7$.

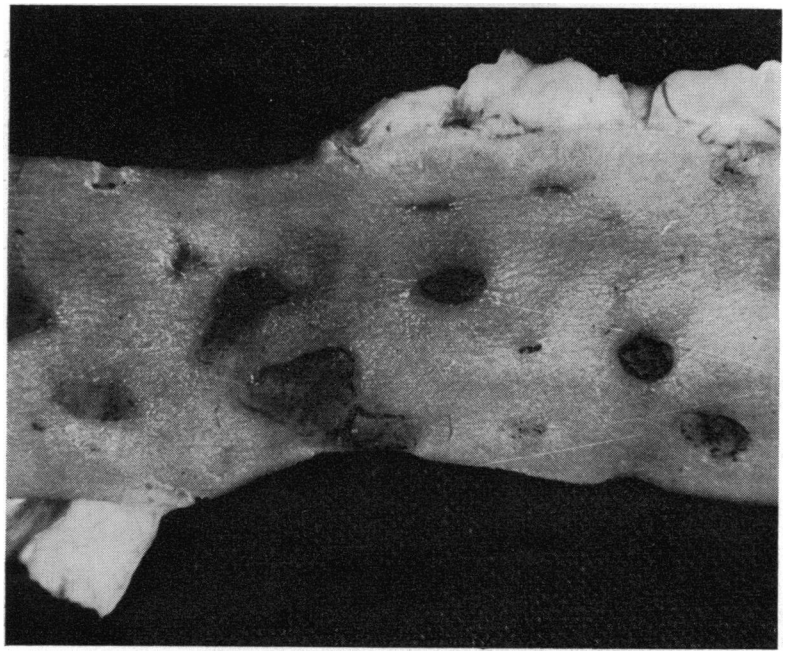

Fig. 3 *Multiple haemorrhagic and non-haemorrhagic ulcers in the rectum* $\times 3{\cdot}8$.

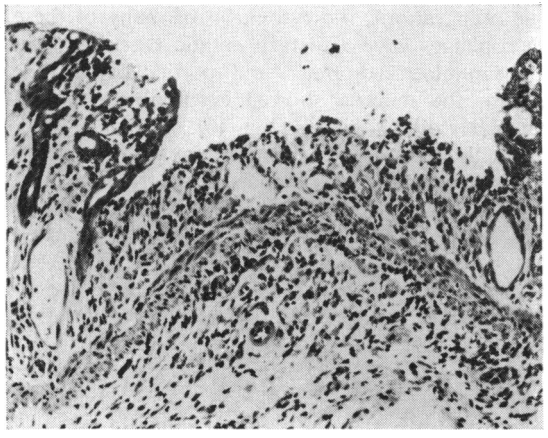

Fig. 4 *Ulceration of the mucosal surface of the colon. The cellular infiltrate in the floor of the erosion includes polymorphonuclear leucocytes and macrophages. The muscularis mucosae is intact. There is oedema of the stroma and dilatation of the glands at the margins of the erosion. Haematoxylin and eosin* ×*130.*

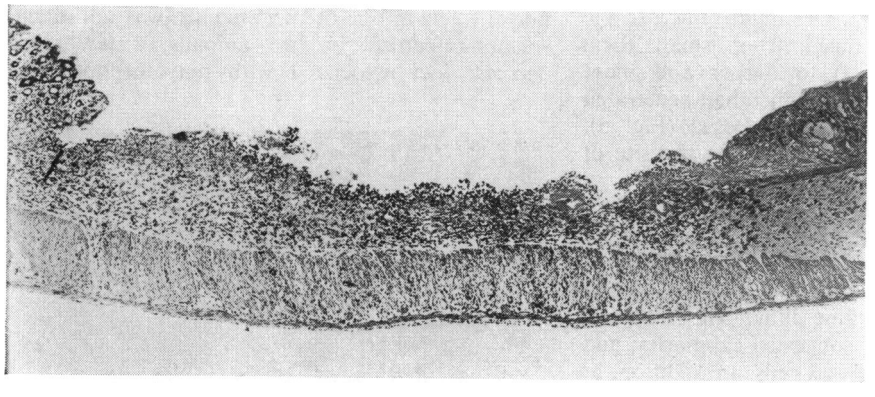

Fig. 5 *Ulceration of the rectum. The muscularis mucosae is breached. The cellular infiltrate extends into the submucosa which is oedematous. Haematoxylin and eosin* ×*30.*

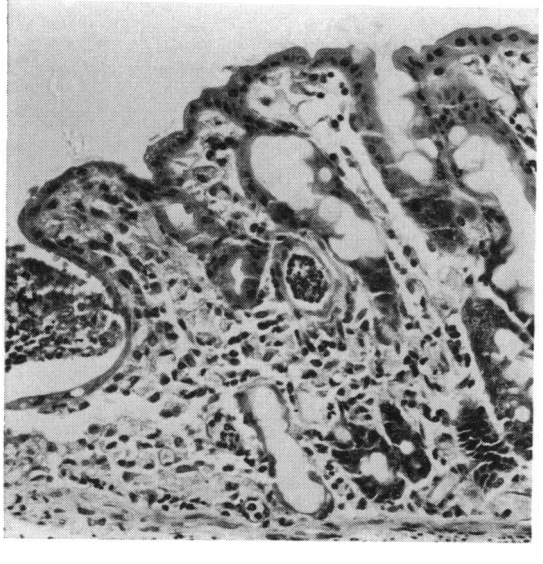

Fig. 6 *Mucosa at ulcer margin showing irregular dilatation of the glands of Lieberkühn and a crypt abscess in the upper part of the mucosa. The stroma is oedematous and infiltrated by macrophages and polymorphonuclear leucocytes. Haematoxylin and eosin* ×*220.*

small areas of mucosal congestion. The ulcers were fewer in number; in some animals only one or two lesions were found.

In the rectum, the bowel wall was frequently thickened and the mesentery thickened and opaque. On direct examination of the mucosa, multiple ulcerative lesions, both haemorrhagic and non-haemorrhagic, were readily seen (Fig. 3). When severe they extended throughout the entire length of this part of the bowel. They varied in shape and size and measured up to 4 or 5 mm diameter, in general being larger than those in the caecum or colon. Small lesions were recognizable by the associated marginal congestion. Very small mucosal ulcerations were observed using a hand lens or sometimes only after microscopic examination.

MICROSCOPIC FEATURES

The ulcerative lesions in the caecum, colon, and rectum presented basically similar microscopic features. Ulcerations ranged from small focal mucosal erosions (Fig. 4) to deeper and more extensive ulcerations, some of which had penetrated and destroyed the muscularis mucosae (Fig. 5). Occasionally ulcerations were noted at the site of lymphoid follicles.

In early lesions the floor and margins were infiltrated with polymorphonuclear leucocytes and macrophages, the cytoplasm of some of the macrophages containing material which stained metachromatically with toluidine blue. The marginal mucosa showed oedema, congested capillaries, and small foci of extravasated red cells. In addition, at the ulcer margins, the glands of Lieberkühn were often irregularly dilated, some showing loss of mucin-secreting cells and degeneration of the lining epithelium, as well as crypt abscesses (Fig. 6).

In older lesions, crypt abscesses were also present at the margins and the cellular infiltrate in the ulcer base included lymphocytes and occasional plasma cells as well as polymorphonuclear leucocytes and macrophages. A cellular infiltrate of similar type in association with oedema was present in the submucosa in relation to ulcers which had penetrated the muscularis mucosae (Fig. 7). Fibroblastic proliferation was noted in the base of some of the older lesions, and ulcers in various stages of healing were found in animals killed on the 35th and 45th days of the experiment.

The intervening unulcerated areas of the large bowel showed occasional very small foci of extravasated red blood cells as well as larger focal cellular infiltrates in the lamina propria, the cells comprising a few lymphocytes and polymorphonuclear leucocytes, and in greater numbers macrophages, often with 'foamy' cytoplasm (Figs. 8 and 9). In toluidine blue preparations, the cytoplasm of many of these macrophages gave a metachromatic reaction. Also in the unulcerated areas, and particularly in the rectum, the mucosa showed crypt abscesses and irregularly dilated glands (Fig. 10).

Paneth cells were not found in any of the sections of the colon in our experimental material obtained from animals fed degraded carrageenan up to 45 days. Sections of the mesentery of the rectum showed that the thickening observed with the naked eye in some of the experimental animals was due to inflammatory reaction and fibroblastic proliferation (Fig. 11).

OTHER ORGANS

Microscopically there was fatty change in the liver in nine of the 14 animals in the experimental group. The fatty change was focal in distribution. Periportal round cell infiltration, although present in a few portal tracts in one or two animals, was not a conspicuous feature. In two animals, a few small necrotic foci associated with polymorphonuclear

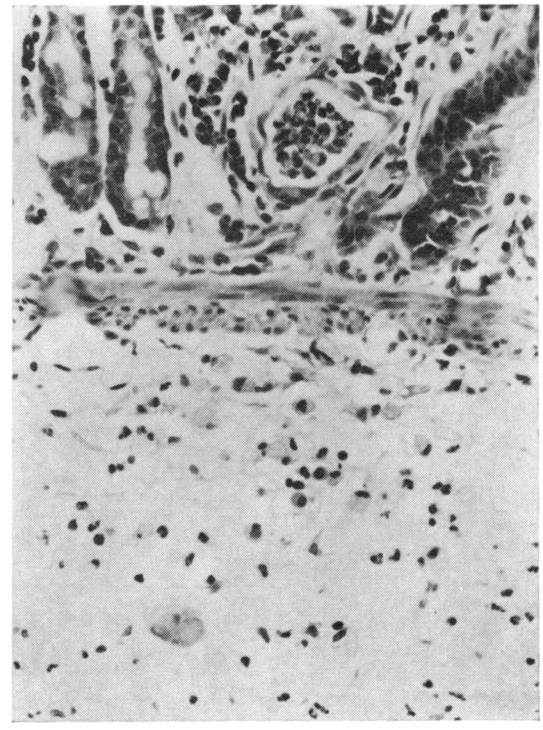

Fig. 7 *Section of colon close to ulcer margin. The mucosal and submucosal infiltrates include macrophages, polymorphonuclear leucocytes, and occasional lymphocytes and plasma cells. Note the crypt abscess in the deeper part of the mucosa. Haematoxylin and eosin* ×*300.*

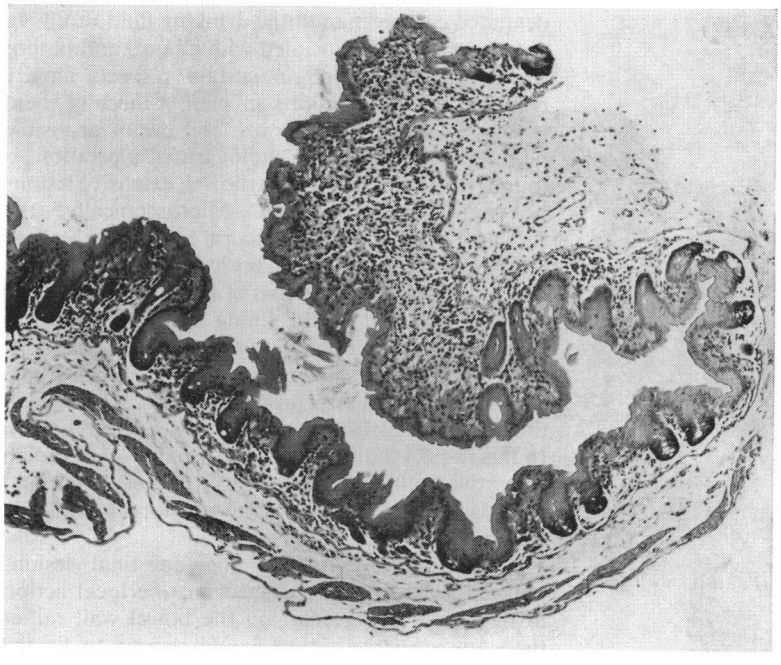

Fig. 8 *Focal cellular infiltration of the mucosa of the caecum in an unulcerated area. Haematoxylin and eosin ×60.*

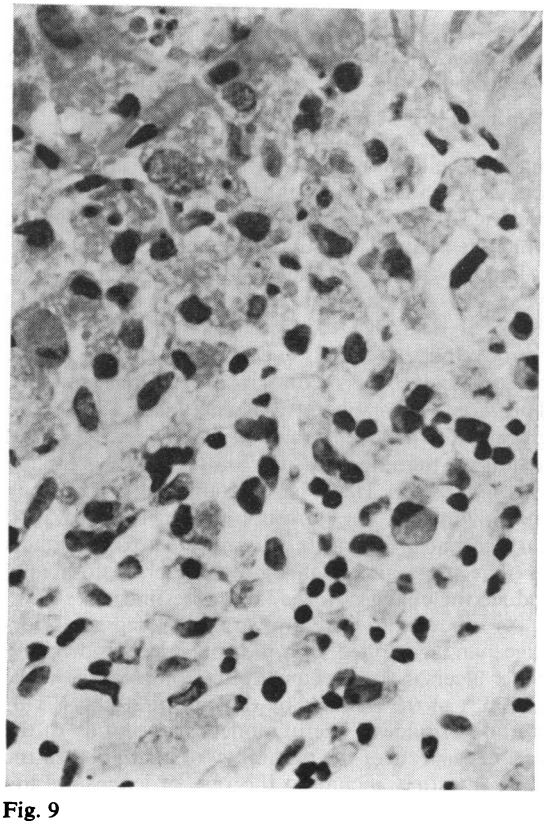

Fig. 9

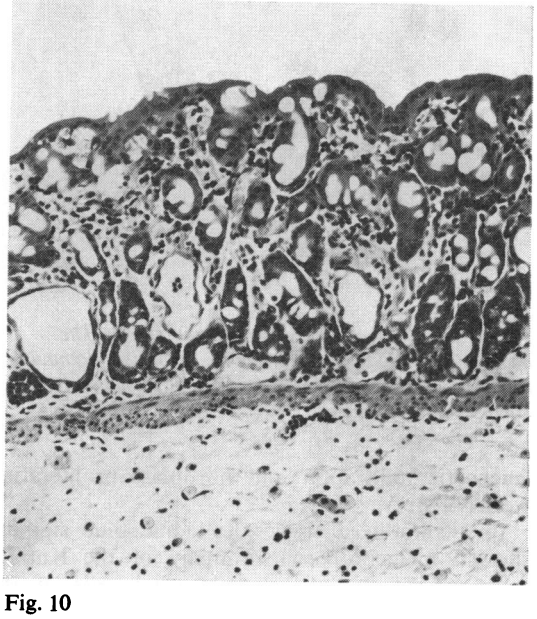

Fig. 10

Fig. 9 *High-power view of mucosal infiltrate shown in Figure 8. The cells include macrophages, some with 'foamy' cytoplasm, polymorphonuclear leucocytes, and a few lymphocytes. Haematoxylin and eosin ×550.*

Fig. 10 *Mucosa of rectum in an unulcerated area showing irregular dilatation of the glands of Lieberkühn. Haematoxylin and eosin ×150.*

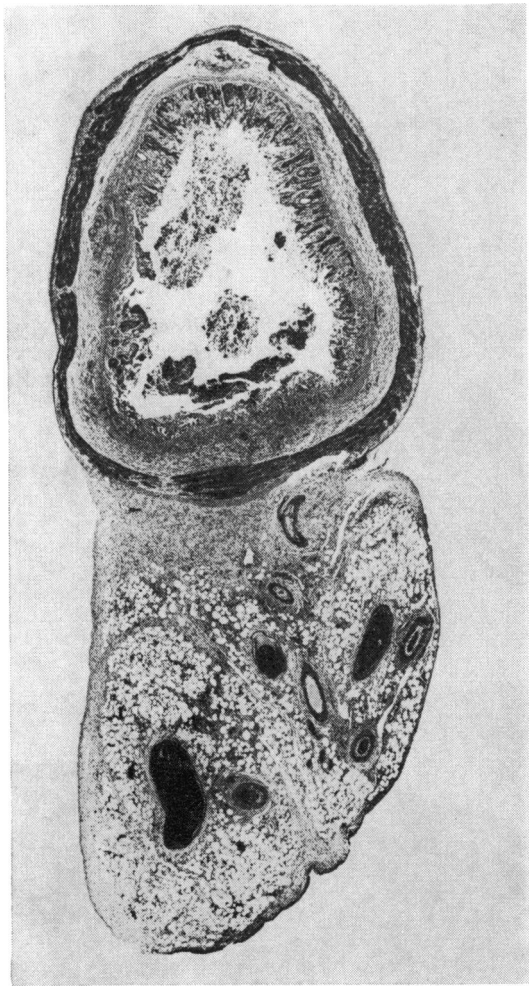

Fig. 11 *Section of rectum and its mesentery. The mucosa shows extensive ulceration. There is inflammatory thickening of the wall of the bowel and the mesentery. Haematoxylin and eosin* $\times 14$.

leucocytic infiltration and fibroblast proliferation were noted.

In alcohol-fixed tissues, toluidine blue staining showed metachromasia in many of the Kupffer cells and in the reticuloendothelial cells of the mesenteric lymph nodes and spleen. Metachromasia was also present in the stroma of the renal pyramids and in some of the epithelial cells of the convoluted tubules and collecting ducts.

Discussion

The above method for the experimental production of ulcers in the large intestine of the guinea pig using degraded carrageenan in the drinking fluid results in ulcerative lesions associated with clinical and pathological changes which in certain respects closely resemble ulcerative colitis in man. Clinically there is loss of weight, loose stools, and occult or visible blood in the faeces. Pathologically, ulceration is found in all parts of the large bowel, extensive lesions occurring in the rectum. Microscopically, the similarities include focal mucosal haemorrhages and cellular infiltrates, oedema, crypt abscesses, irregular dilatation of crypts with loss of mucin-secreting cells and degeneration of the lining epithelium, ulceration involving mainly the mucosa, as well as ulcerations in various stages of progression and healing. The ulcerative lesions, however, appear to begin in the caecum and extend distally toward the rectum. In this respect it differs from the usual type of human ulcerative colitis which begins in the left colon and extends proximally. It also differs histopathologically by the absence of Paneth cells.

In the pathogenesis of the intestinal lesions induced by degraded carrageenan, the local action of degraded carrageenan on the bowel wall rather than any systemic action would appear to be the important factor. It is probable that some of the degraded carrageenan is absorbed: this would explain the presence of toluidine blue metachromatic material in macrophages in the mucosa of the large intestine, in reticulo-endothelial cells of the mesenteric lymph nodes, liver, and spleen, in the stroma of the renal pyramids, and in the urine. The focal mucosal cellular infiltrates observed in unulcerated areas may indeed represent the local reaction to the presence of degraded carrageenan within the mucosa which at a later stage undergoes ulceration.

During gastric secretory studies in guinea pigs, we have administered parenterally much larger doses of degraded carrageenan over a prolonged period and have not encountered ulcers in the caecum, colon, or rectum in any of our animals. This would also suggest that degraded carrageenan given orally produces its damaging effect by a local action on the intestinal mucosa rather than by any systemic effect. There is, of course, the possibility that hydrolysis or fermentation of the degraded carrageenan by bacteria may release toxic products or that some alteration of the bacterial flora may induce the changes in the large intestine.

As our results have shown, when degraded carrageenan is supplied to guinea pigs in their drinking water ulcers in the caecum, colon, and rectum occur in 100% of the animals by the end of 30 days. This method provides a useful model for the study of the various aspects of the pathology of ulceration in the large intestine, including the rate of extension and healing of ulcers, bacterial changes in the colon,

electrolytic changes in the blood, immunological responses, and other possible systemic effects. In addition the method may be adapted for the investigation and assessment of the therapeutic effects of various drugs and other forms of treatment.

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References

Kirsner, J. B. (1961). Experimental 'colitis' with particular reference to hypersensitivity reactions in the colon. *Gastroenterology*, **40**, 307-312.

Marcus, R., and Watt, J. (1969). Seaweeds and ulcerative colitis in laboratory animals. *Lancet*, **2**, 489-490.

Watt, J., and Marcus, R. (1965). Méthode de coloration macroscopique des polysaccharides sulfatés au niveau de la muqueuse gastrique. *Path. et Biol.*, **13**, 961-962.

Zweibaum, A., Morard, J. C., and Halpern, B. (1968). Réalisation d'une colite ulcéro-hémorragique expérimentale par immunisation bactérienne. *Path. et Biol.*, **16**, 813-823.
