did not alter responses, to noradrenaline, of vasa from guanethidine-pretreated animals.

During passage along the gut it is possible that a compound such as hordenine could be metabolized by microsomal enzymes which catalyse reactions such as *N*-demethylation (Lindeke & Cho 1982) and glucuronide formation (Mulder 1982). The activity of these enzymes is generally low in the intestine (Hanninen et al 1987) and in the rat, where hepatic-microsomal enzyme activity is higher than in intestine, hepatic-microsomal metabolism of hordenine was not detectable (Barwell et al 1984). Dietary-hordenine might be deaminated by MAO, which is present in the epithelium in high calalytic activity. However, more than 90% of this activity is due to MAO-A (Barwell & Canham 1988) which this study has shown does not deaminate hordenin. Therefore, intestinal deamination of hordenine would be dependent upon MAO-B. Studies with selective irreversible inhibitors of MAO-A have shown that intestinal MAO-B activity is apparently insufficient to prevent absorption of oral-tyramine (Youdim 1977). Therefore, it is unlikely that intestinal MAO-B activity is sufficient to deaminate dietary-hordenine, which is likely to be absorbed. Under the in-vitro conditions of this investigation hordenine produced a significant effect, upon responses to noradrenaline, at 25 μM. This was similar to the concentration of tyramine (50 μM) which produced half maximum contractions of the vasa. Therefore, like tyramine, dietary hordenine is likely to produce adverse pharmacological effects, upon the sympathetic nervous system.

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Rapid production of ulcerative disease of the colon in newly-weaned guinea-pigs by degraded carrageenan

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Abstract—In a dose-response study, degraded carrageenan (*Eucheuma spinosum*) was supplied in the drinking fluid at 1,2 and 3% concentrations over two weeks to young adult guinea-pigs. Ulceration of the large bowel was produced in 100% of animals, the severity and extent of damage probably being dose-related. In a time-course study, 3% degraded carrageenan solution supplied to newly-weaned guinea-pigs produced in 100% of animals ulceration in the caecum by four days and in the ascending colon by seven days. The onset of ulceration occurred as early as the second day. This model is convenient and economic for the screening of drugs of potential therapeutic value in human ulcerative colitis.

An experimental model for the investigation of ulcerative disease of the colon has become available in recent years following the recognition that certain sulphated polysaccharides fed to a variety of animal species will produce ulceration of the large bowel in a high proportion of animals (Marcus & Watt 1969; Mottet 1972; Watt & Marcus 1973). One of the most active ulcerogenic agents is carrageenan, derived from the red seaweed *Eucheuma spinosum*. When carrageenan, either in its native form or as a degraded product, is supplied in the drinking fluid, ulceration of the colonic mucosa occurs after a period which may range from about two weeks to as long as three months or more, depending on the nature and concentration of carrageenan supplied and the particular animal species used (Watt & Marcus 1973). In studying the effects of pharmacological and therapeutic agents in relation to experimental ulcerative disease of the colon, there are distinct advantages in having an animal model in which ulceration begins within a few days rather than weeks.

In this paper we describe a dose response in young guinea-pigs and a time-course study in newly-weaned animals, the results of which provide a convenient experimental model for the rapid production of carrageenan-induced ulcerative disease of the colon in 100% of animals by four days.

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Table 1. Incidence, severity and extent of ulceration of the large bowel in young adult male guinea-pigs (10 per group) fed 1%, 2% and 3% degraded carrageenan solutions as drinking fluid for a period of two weeks.

Conc. of deg. carrageenan sol. supplied as drinking fluid	Average daily intake of degraded carrageenan (g/100 g)	Incidence of ulceration	No. of animals with ulceration of graded severity*					No. of animals with ulceration at each site within the large bowel			
			1+	2+	3+	4+	5+	Caecum	Ascending Colon	Transverse & Distal Colon	Rectum
1%	0·2	100%	1	4	2	0	3	10	6	0	0
2%	0·3	100%	0	2	2	2	4	10	8	0	0
3%	0·4	100%	0	0	0	0	10	10	10	5	4

* Severity assessed according to the total number of ulcers found in the large bowel as follows:—1+ = 1–50, 2+ = 51–200, 3+ = 201–350, 4+ = 351–500, 5+ = over 500 ulcers.

Materials and methods

Supplies of food quality powdered extract of native or undegraded carrageenan derived from the red seaweed *Eucheuma spinosum* were obtained from British Ceca Company Ltd. (London). The dry powder was degraded by exposure to concentrated HCl for 1 h, as in the method previously described (Watt et al 1979).

Young adult and newly-weaned male albino guinea-pigs (Dunkin-Hartley strain) were obtained from an accredited source (Redfern Animal Breeders Ltd). Their diet consisted of vitamin C-enriched cubes (RGP-Dixon and Sons Ltd) and drinking bottles were fitted with metal ball-valve teats which limited the amount of spillage. Daily fluid intakes were measured and feeding bottles replenished so that drinking fluid was always available.

All animals were weighed at the start of each experiment and again at the end of one week and two weeks according to the length of the experimental period. Weight changes were expressed as the mean and standard deviation of the animals in each group, and simple t-tests for statistical analysis were applied. Animals were killed by a sharp blow on the head followed immediately by transection of the neck vessels. The large bowel was removed and damage assessed according to the method described below.

An initial dose-response experiment was performed in young adult guinea-pigs over two weeks. Forty guinea-pigs (450–500 g) were randomized into four groups of ten animals each. One group was supplied 1%, a second group 2% and the third group 3% degraded carrageenan solutions as drinking fluid. A control group received only water as drinking fluid. At the end of the two weeks the incidence, severity and extent of ulceration of the large bowel in each of the three experimental groups were assessed and the results compared.

In a time-course study, 24 newly-weaned guinea-pigs (200–250 g) were randomized into six groups of four animals each. They were housed in pairs and received as drinking fluid 3% degraded carrageenan solution over a period of seven days. One group of four animals was killed on each successive day, from the second day, until the end of the experiment. The incidence, severity and extent of ulceration of the large bowel in each of the six groups were then assessed.

At necropsy, the large bowel including rectum was removed along with a 5 cm segment of ileum. A 4% solution of formaldehyde in normal saline was injected at the proximal and distal ends until the lumen of the bowel was moderately distended. After 24 h fixation the specimen was opened by a longitudinal incision which in the caecum was carried through the major curvature midway between the dorsal and ventral longitudinal taeniae, in the ascending colon through the antimesenteric border, and in the transverse colon, descending colon and rectum, through the line of the mesenteric attachment. The bowel was thoroughly washed in running water and then examined by transmitted light using a Burkard 'cold light' viewer with a magnifying lens attachment (10 diopters). The presence or absence of ulceration of the large bowel was noted. Ulcers were then carefully counted and their distribution charted with particular reference to the caecum, ascending, transverse and descending colon, and rectum. The severity of damage was assessed according to the total number of ulcers found in the large bowel, as follows: 1+ = 1–50, 2+ = 51–200, 3+ = 201–350, 4+ = 351–500, 5+ = over 500 ulcers.

Results

The degraded carrageenan solutions were readily accepted as drinking fluid by all of the guinea-pigs. Some looseness of the stools developed in most of the animals after a few days. Control animals given water only as drinking fluid showed body weight gains of $40{\cdot}9 \pm 32$ g by the end of the first week and $43{\cdot}8 \pm 11{\cdot}5$ g by the end of the second week. Animals which received the lower concentrations viz. 1% and 2% degraded carrageenan as drinking fluid gained $36{\cdot}8 \pm 44{\cdot}2$ g and $40{\cdot}3 \pm 33{\cdot}7$ g, respectively during the first week, and lost $25{\cdot}4 \pm 74{\cdot}3$ g ($P < 0{\cdot}01$) and $15{\cdot}5 \pm 77{\cdot}4$ g ($P < 0{\cdot}05$), respectively by the end of the second week. Animals fed the highest concentration viz. 3% degraded carrageenan showed a significant weight loss of $32 \pm 74{\cdot}5$ g ($P < 0{\cdot}01$) by the end of the first week and $52{\cdot}9 \pm 27$ g ($P < 0{\cdot}01$) by the end of the second week.

In the dose-response study the young adult guinea-pigs which received 1%, 2% and 3% degraded carrageenan solution as drinking fluid over the two week period drank on average 93, 72, and 66 mL per animal per day, respectively; allowing for spillage, the corresponding average maximal daily intake of degraded carrageenan per animal in each of these groups was 0·2, 0·3 and 0·4 g per 100 g body weight. The incidence, severity and extent of ulcerative disease of the large bowel in each of these three groups of young adult animals are shown in Table 1. Ulceration of the large bowel was present in all animals in each group, the severity and extent of damage approximately corresponding to the average maximal daily intake of degraded carrageenan. In guinea-pigs fed the higher concentrations (2% and 3%) of degraded carrageenan, ulceration of the caecum and ascending colon was more severe and more extensive than in animals receiving the lowest concentration (1%). Among the guinea-pigs fed 3% degraded carrageenan solution, ulceration extended into the distal colon and rectum. There was no ulceration in any of the control animals.

Table 2 shows the results of a time-course study in which the development of ulceration of the large bowel was followed at daily intervals from two to seven days in newly-weaned male albino guinea-pigs supplied 3% degraded carrageenan as drinking fluid. Ulceration of the caecum was observed in one of the four animals killed at the end of two days. The incidence of ulceration increased to 100% in the caecum by four days and in the ascending colon by seven days (Figs 1, 2). There was no ulceration in the remainder of the large bowel including rectum

Table 2. Time-course study. Incidence, severity and extent of ulceration of the large bowel in newly-weaned male guinea-pigs (four per group) fed 3% degraded carrageenan solution as drinking fluid for two to seven days.

Duration of experiment (days)	Average daily intake of degraded carageenan (g/100 g)	Incidence of ulceration	No. of animals with ulceration of graded severity*					No. of animals with ulceration at each site within the large bowel		
			1+	2+	3+	4+	5+	Caecum	Ascending Colon	Transverse & Distal Colon and Rectum
2	0·57	1/4	1	0	0	0	0	1	0	0
3	0·57	3/4	2	1	0	0	0	3	0	0
4	0·60	4/4	0	2	0	1	1	4	2	0
5	0·46	4/4	0	1	2	0	1	4	2	0
6	0·55	4/4	0	0	1	0	3	4	3	0
7	0·57	4/4	0	0	0	0	4	4	4	0

* Severity as assessed in Table 1

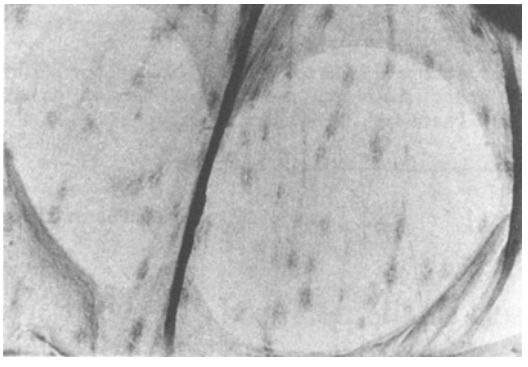

FIG. 1. Multiple ulceration of caecum in a newly-weaned guinea-pig fed 3% degraded carrageenan solution as drinking fluid for 4 days. Ulcerated lesions show central pallor with darker margins. Specimen viewed by transmitted lighting. M × 1·5.

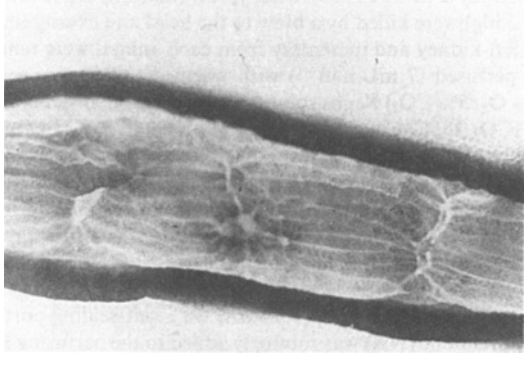

FIG. 2. Three irregular ulcers in ascending colon of newly-weaned guinea-pig fed 3% degraded carrageenan solution for 4 days. Specimen viewed by transmitted lighting. M × 5·3.

in any of the newly-weaned animals. Histology of the acute lesions in caecum and ascending colon showed ulceration, cellular infiltrates comprising macrophages and polymorphonuclear leucocytes, dilatation of glands, and crypt abscesses (Fig. 3).

Discussion

Our results confirm the high ulcerogenicity of degraded carrageenan prepared from whole extracts of the red seaweed *Eucheuma spinosum*, ulceration of the large bowel being readily produced in newly-weaned and young adult guinea-pigs. This is in contrast to the negative results reported by others who have investigated the ulcerogenic effects of degraded and non-

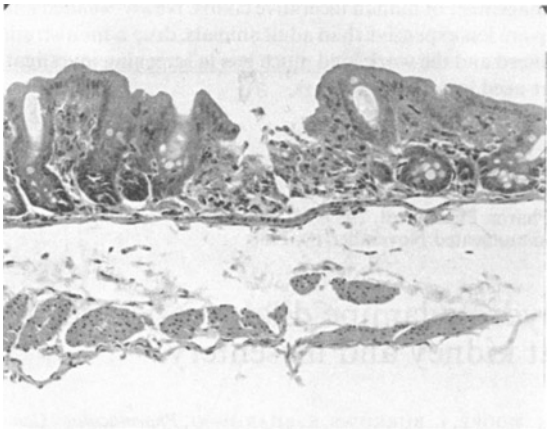

FIG. 3. Ulceration of the mucosa of the caecum in a newly-weaned guinea-pig fed degraded carrageenan for 4 days. The cellular infiltrate includes macrophages and polymorphonuclear leucocytes; adjacent glands are dilated. Section (5 microns) stained by haematoxylin and eosin. M × 180.

degraded, fractionated, carrageenans (iota-type) derived from the same seaweed, *Eucheuma spinosum* (Norris et al 1981).

The dose response study showed that even a 1% degraded carrageenan solution supplied as drinking fluid for two weeks caused ulcerative disease of the large bowel in all of the young adult male guinea-pigs. Allowing for equal amounts of spillage in the various experimental groups, there was some degree of correspondence between the severity and extent of ulceration and the maximal average daily intake of degraded carrageenan on a body weight basis. The results suggest that the severity and extent of carrageenan-induced damage in the guinea-pig colon may be dose-related.

The dose response study would also suggest that the sequence in the development of ulceration along the large bowel is that ulceration begins in the caecum and extends caudally into the ascending colon, transverse colon, distal colon and rectum. In this respect, the site of onset and progression of the disease as observed macroscopically differ from carrageenan-induced ulceration in the rat (Fabian et al 1973) and from the more usual type of human ulcerative colitis, both of which are considered to begin in the distal colon and rectum.

The time-course investigation in newly-weaned guinea-pigs has shown that 100% incidence of ulceration occurs in the caecum by four days and in the ascending colon by seven days. Ulceration in the caecum begins to develop even as early as the second day and in the ascending colon by the fourth day. The histopathological features of the ulcerative lesions in the newly-

weaned guinea-pigs are similar to those in adult animals and include mucosal ulceration, inflammatory cell infiltration, dilatation of glands, and crypt abscesses, features which are also seen in human ulcerative colitis (Watt & Marcus 1971).

The assessment of the effect of degraded carrageenan on guinea-pigs, and its use as an animal model in the screening of drugs, must take into consideration factors such as weight loss, alteration of bowel function, as well as damage within the large bowel. The method of assessment of damage to the bowel may be on an arbitrary basis according to the grading system which we have used because of its practical value; this also includes the extent of involvement from caecum to rectum. Alternatively a more strict numerical assessment of ulceration, with or without histology, may be preferred,

An experimental model of this kind, with a relatively rapid onset of ulceration, provides a convenient and economic method for evaluating drugs of potential therapeutic value in the management of human ulcerative colitis. Newly-weaned guinea-pigs are less expensive than adult animals, drug administration is reduced and the work load much less in screening investigations that need last only four days.

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Hydroxylamine dilates resistance blood vessels of the perfused rat kidney and mesentery

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Abstract—Hydroxylamine (ED50 values, 47 ± 8·9 nmol and 320 ± 39 nmol) dilates resistance arterioles of the perfused noradrenaline-preconstricted rat kidney and mesentery. In this respect hydroxylamine was approximately 63 × and 320 × less potent than acetylcholine (ACh) and 15 × and 128 × less potent than nitroprusside in the two perfused organs studied. The vasodilator effect of hydroxylamine (unlike that of ACh) was unaffected by CHAPS de-endothelialization suggesting that its effect is independent of endothelium-derived relaxing factor (EDRF).

In recent years considerable interest has been shown in the mechanism of action of nitrovasodilators such as nitroprusside on vascular smooth muscle. This research has received added impetus following the recent discovery that endothelium derived relaxing factor (EDRF) is identical with nitric oxide (NO) (Palmer et al 1987) which may thus be considered the body's 'endogenous nitrovasodilator'. Hydroxylamine is both a natural product of mammalian cells (Gross 1985) and a potential source of NO under appropriate experimental conditions (see Waldman & Murad 1987 for review). In addition, hydroxylamine has been reported to cause dose-related relaxation of the mouse isolated anococcygeus preparation (Gibson & Mirzazadeh 1988). For this reason we decided to investigate the potential vasodilator effect of this substance on resistance blood vessels of the perfused rat kidney and mesentery.

Materials and methods

The procedures used have been described in detail elsewhere (Bhardwaj & Moore 1988). Briefly, rats (male, Sprague-Dawley, 250–350g) were killed by a blow to the head and exsanguinated. The left kidney and mesentery from each animal were removed and perfused (7 mL min^{-1}) with warmed (37°C), oxygenated (95% O_2:5% CO_2) Krebs solution (composition, mM: NaCl 118, $NaHCO_3$ 25, $CaCl_2$ 1·9, $MgSO_4$ 1·19, KCl 4·75, KH_2PO_3 1·19, glucose 11·1, pH 7·2) via cannulae inserted into the aorta and superior mesenteric artery respectively. Indomethacin (7 μM) was routinely added to the perfusing Krebs solution to inhibit vascular prostacyclin (PGI_2) biosynthesis. Perfusion pressure was constantly monitored by means of a Bell & Howell pressure transducer connected to a Devices pen recorder. Drugs were injected in small volumes (< 10 μL) via a self-sealing port. (−) Noradrenaline (NA) was routinely added to the perfusing Krebs solution at a concentration (0·1–0·5 mM, kidney; 100–250 μM, mesentery) which produced approximately 60–80% of the maximum response. Kidneys and mesenteries were weighed before and at the end of each experiment to determine the extent of oedema formation. In some experiments, endothelial cells lining resistance blood vessels were removed by infusion (30 s) of 4·7 mg mL^{-1} 3,3 cholamidopropyl dimethylammonio 1-propanesulphonate (CHAPS).

All drugs were purchased from Sigma Ltd, dissolved in saline (0·9% w/v NaCl) and kept on ice throughout the experiment. Drug solutions were prepared fresh each day. Results show mean ± s.e. mean with the number of observations indicated in parentheses. Statistical significance of differences between groups was determined using unpaired Student's *t*-test.

Results

Basal perfusion pressure of kidney and mesentery preparations

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