Patients treated with indomethacin have developed gastrointestinal symptoms such as nausea, vomiting, dyspepsia, peptic ulcer and gastrointestinal haemorrhage, particularly when large doses were given (Lövgren & Allander, 1964; Ballabio, 1965; Rothermich, 1966). Anderson (1965) found that indomethacin can cause gastric erosion and haemorrhage in starved guinea-pigs. In dogs, Nicoloff (1968) observed that the administration of indomethacin induced antral and gastric ulceration with melena and perforation, as well as jejunal ulcers.

Our investigations show that in rats the jejunum is most susceptible to the ulcerogenic effect of indomethacin. The pathogenesis of jejunal ulcers still needs elucidation and it is felt that this easily reproducible experimental model may facilitate the study of factors influencing jejunal ulcers in rats.

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Effect of subcutaneously administered degraded carrageenan on the production of histamine-induced gastric and duodenal ulceration

We recently reported the pronounced and prolonged inhibitory effect of parenterally administered degraded carrageenan on the acid gastric secretory response of the guinea-pig to histamine (Eagleton, Watt & Marcus, 1968). We have now made a comparison of the protection afforded by parenteral carrageenan against histamine-induced gastric ulceration and histamine-induced duodenal ulceration in the same species.

For the selective production of gastric and duodenal ulcers, histamine acid phosphate was given to fasted adult male albino guinea-pigs, 550–650 g, and the lesions evaluated (Eagleton & Watt, 1965, 1967). Freshly prepared degraded carrageenan (5% aqueous solution) derived from the red seaweed *Eucheuma spinosum* was given as a single subcutaneous injection, 400 mg/kg, to all test animals 12 h before administration of histamine; control animals received no carrageenan. For the production of gastric ulcers, the animals were injected intraperitoneally with 5 mg of histamine (doses refer to the salt) per kg and killed 3 h later, i.e. 15 h after receiving carrageenan. For the production of duodenal ulcers, 8 injections of 0·25 mg histamine/kg were given intramuscularly at $\frac{1}{2}$ h intervals; the animals were killed 4 h after the first injection of histamine, i.e. 16 h after receiving carrageenan.

We also investigated the effect of degraded carrageenan in doses ranging from 195 to 550 mg/kg on the incidence and severity of histamine-induced duodenal ulceration, the carrageenan being injected 12 h before histamine. The volume and total acid concentration (titration with phenolphthalein as indicator) of the gastric juices removed at autopsy were measured.

Table 1. *Effect of* 12 *h pretreatment with subcutaneously administered degraded carrageenan on histamine-induced gastric ulceration*

Group	Incidence of gastric ulceration	No. o; animals with gastric lesions of severity† ±	+	++	+++
Control (7)*	100%	0	0	4	3
	P > 0·30				
Test (15)	87%	1	4	8	0

* No. of animals.
† Arbitrary scale. ± = Tiny areas of epithelial loss just visible to the naked eye. +++ = Numerous extensive areas of epithelial loss involving at least 2 of the three divisions of the stomach.

Table 2. *Effect of* 12 *h pretreatment with subcutaneously administered degraded carrageenan on histamine-induced duodenal ulceration*

Group	Duodenal ulceration: Incidence	No. with	No. without	Mean length of duodenum involved (cm)
Control (14)*	100%	14	0	8·5 ± 1·7
	P < 0·01			P < 0·01
Test (20)	55%	11	9	0·3 ± 0·6

* No. of animals.

Table 1 shows the effect of degraded carrageenan on the production of gastric ulceration. There was slight reduction in the incidence of ulceration, 2 animals being completely protected. Of the 13 animals with ulceration, 5 presented lesions of low severity.

The effect of degraded carrageenan on the production of duodenal ulceration is shown in Table 2. Both the incidence of ulceration and the severity of damage were greatly reduced.

In the dose-response study, at 7 dose levels below 400 mg/kg the incidence of duodenal ulceration was 56% and the average length of duodenum involved was 0·6 cm. At 6 dose levels at and above 400 mg/kg, the incidence of ulceration was 17% and the average length of duodenum involved was 0·2 cm. In all the animals in the dose-response study, the gastric juice volumes at autopsy were 1 ml or less and the total acid concentrations ranged from 43 to 79 m-equiv/litre, values which are below fasting for the guinea-pig. In control animals, juice volumes ranged from 9 to 12 ml and total acid concentrations from 105 to 112 m-equiv/litre.

From these results it is evident that subcutaneously administered degraded carrageenan offers marked protection against histamine-induced duodenal ulceration. This is in contrast to antihistamines which show no protective action against such lesions (Watt & Eagleton, 1966). On the other hand, the degree of protection afforded by antihistamines against histamine-induced gastric ulceration (60% protection) is much greater than that afforded by carrageenan in the above study (13% protection) (Watt & Eagleton, 1964).

In animals given parenteral degraded carrageenan, repeated tests have failed to demonstrate the presence of toluidine blue metachromasia either in the fasting or

histamine-stimulated gastric secretions. The absence of detectable amounts of degraded carrageenan in the gastric or duodenal contents indicates that the protection against histamine-induced duodenal ulceration afforded by parenteral carrageenan is not attributable to a local antipeptic action. On the other hand, in contrast to antihistamines which neither suppress histamine-stimulated acid secretion nor reduce the severity of histamine-induced duodenal ulceration, parenterally administered carrageenan both suppresses acid secretion and has a marked protective effect against histamine-induced duodenal ulceration. It is reasonable to suppose, therefore, that this inhibition of acid gastric secretion by systemic carrageenan is a major factor in preventing histamine-induced duodenal damage.

The sulphated polysaccharides are in general classified as antipeptic agents and their role in preventing experimental gastroduodenal ulceration as well as in the therapy of peptic ulceration in man is largely attributed to this property (Houck, Bhayana & Lee, 1960; Sun, 1967). From our own observations we consider that the systemic anti-secretory action of sulphated polysaccharides, in particular degraded carrageenan, is an important aspect of their pharmacological activity that should not be ignored.

In several species, viz. rabbit, rat and guinea-pig, we have observed that when degraded carrageenan—and indeed even a crude aqueous extract of the seaweeds *Chondrus crispus* or *Eucheuma spinosum*—is given orally, both the urine and faeces show the presence of metachromatic staining material demonstrable by toluidine blue. Similarly staining material is also present in macrophages in colonic mucosa and mesenteric lymph nodes, in Kupfer's cells in the liver, as well as epithelial cells of the convoluted tubules and collecting ducts of the kidney. This is strong evidence that some of the degraded carrageenan is absorbed into the blood stream, as Anderson & Soman (1963) had already suspected from their studies in the guinea-pig. It may be, therefore, that apart from any local action in the stomach, degraded carrageenan when given orally is absorbed from the bowel and thereafter exerts a systemic anti-acid secretory and anti-ulcer effect, either by complexing with histamine or by stimulating the release into the blood stream of diamine oxidase as occurs in response to the sulphated polysaccharide heparin (Dahlbäck, Hansson & others, 1968). In retrospect, this could adequately explain the inhibition of histamine-stimulated gastric secretion observed in guinea-pigs fed oral carrageenan over a 2 week period (Anderson, Marcus & Watt, 1962). Similarly, such a systemic anti-acid secretory action could also account for the protection against histamine-induced duodenal ulceration which Houck & others (1960) observed in dogs fed carrageenan over a 30 day period.

Recently, it has been shown both in experimental animals and in man that the sulphated polysaccharide heparin given intravenously has an inhibitory action on acid gastric secretion (Thompson, Lerner & Musicant, 1966; Watt, Eagleton & Marcus, 1966). This inhibitory action in man affects basal secretion as well as stimulated secretion. Heparin's anticoagulant properties, however, would contra-indicate its therapeutic use in the management of patients with peptic ulceration. Such contraindication would not apply to degraded carrageenan (Anderson & Duncan, 1965). Nor is there any granulomatous reaction at the site of injection of degraded carrageenan as occurs with the undegraded preparation. The therapeutic possibilities of parenterally administered sulphated polysaccharides, whether synthetic or naturally occurring, would appear to warrant further exploration, particularly in relation to duodenal ulceration.

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Parallel assay of prostaglandin-like activity in rat inflammatory exudate by means of cascade superfusion

Pharmacological activity was found in inflammatory exudates from rats treated with carrageenan, and was predominantly attributable to the presence of E-type prostaglandins. Some of this work has been briefly reported to the British Pharmacological Society (Willis 1968). Novel features of the experimental methods used are presented here in more detail.

Cascade superfusion for the detection and assay of prostaglandins has been used previously (Ferreira & Vane, 1968; Gilmore, Vane & Wyllie, 1968). However the modifications described below allow reduced flow rates and thus increased sensitivity for the repeated parallel assay of small amounts of prostaglandin-like activity.

Isolated tissues were suspended in chambers formed from non-wettable polypropylene and superfused in series with a stream of Tyrode solution delivered at constant rate by a roller pump (Watson Marlowe MHRE). Tyrode in the reservoir was gassed with air and contained atropine (10^{-6}M), mepyramine (10^{-6}M) and methysergide bimaleate (5×10^{-7}M). The entire cascade was enclosed in a Perspex-fronted box maintained at near 37° and saturated with water vapour from a humidifying device (Fig. 1). This arrangement permitted prolonged survival of up to four tissues with superfusion rates of only 2 to 4 ml/min. Standard prostaglandins (PGs) and extracts in Tyrode solution (0·8 ml or less) were injected into the inlet side of the silicone rubber roller pump tubing. Responses of the tissues were recorded on a kymograph by pendulum levers (Paton 1957) with lengthened writing arms (Schild 1947).

The principal isolated tissue used was the rat stomach strip as prepared by Vane (1957). It was found that a prolonged settling period of several hours resulted in a steady base-line and high sensitivity to prostaglandins (0·5 to 1 ng of PGE_2). This tissue was often used in conjunction with the chick rectum (Mann & West, 1950, Ferreira & Vane, 1967). It was found that under the conditions described, the Tyrode superfused rectum from chicks of 150–200 g responded in a selectively sensitive manner to E-type prostaglandins. This tissuewhile virtually equi-