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Degradation of carrageenan for the experimental production of ulcers in the colon

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Ulcerative disease of the colon can be produced in several laboratory animal species by the oral administration in the drinking fluid of sulphated polysaccharides such as sulphated amylopectin and certain red seaweed extracts including carrageenan (Marcus & Watt 1969; Watt & Marcus 1973). As a result, it is now possible to investigate experimentally the effects of pharmacological and pharmaceutical preparations in relation to ulcerative disease of the colon.

Degraded carrageenan derived from the red seaweed *Eucheuma spinosum* is one of the most potent agents for the production of ulcers in the colon. This product, however, is not readily available commercially and is expensive. The native or undegraded carrageenan from the same seaweed, although readily available and relatively inexpensive, is less ulcerogenic than the degraded product and is more difficult to administer because of its high viscosity. This has inevitably limited the use of carrageenan for the experimental production of ulcers in the colon.

We describe a simple method for degrading carrageenan which can be quickly, conveniently and economically carried out in any gastrointestinal research laboratory. The method relates specifically to the preparation of a 2% degraded carrageenan solution which is acceptable as drinking fluid and causes experimental colonic ulceration within a short time.

Carrageenan extracts. Supplies of the dried extracts of native *Eucheuma spinosum* carrageenan were obtained from two commercial sources, ·viz. British Ceca Company Ltd (London) and Blandola Company Ltd. (Whaley Bridge, Stockport). The carrageenan extract supplied by the British Ceca Co. was a food quality preparation consisting of a fine creamy-white powder, the particle size 98% less than 250 μm. The dry crude extract obtained from Blandola Co. was a creamy powder, the particle size less than 600 μm.

Degradation of carrageenan. After a preliminary trial of several methods for degrading sulphated polysaccharides including exposure to strong acid or alkali, mild acid hydrolysis or oxidation by sodium hypochlorite (Black et al 1965), and u.v. radiation (Emerson & Kerkut 1974), we adopted the described procedure because of its inherent simplicity and effectiveness.

The preparation of the degraded carrageenan involves a brief exposure of the dry undegraded extracts to the action of concentrated HCl. The dry powder is placed in a glass beaker and HCl added and thoroughly mixed at room temperature (20 °C) with a glass rod over 1 h. A 2% degraded carrageenan solution is prepared by

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adding to each gram of the dry powder 1 ml of concentrated HCl. At the end of 1 h, water is added and the mixture stirred and warmed if necessary (35 °C) until all the powder is dissolved. The acidified solution is neutralized with 2M sodium hydroxide to pH 7 to 8 and the volume adjusted to give 2% concentration.

The degraded carrageenan solutions are amber coloured clear fluids, which can be freshly prepared each day or stored in bulk in a refrigerator or cold room for up to one week. Although slightly viscous, the solutions can be administered as a drinking fluid using standard glass feeding bottles without causing blockage of the metal teats. The specific viscosities (Technico BSU viscometer size C) of the undegraded and degraded carrageenan solutions (0·05%) of the food quality carrageenan (British Ceca Co.) are 2·27 and 0·09 respectively; the corresponding values for the solutions of the crude extract (Blandola Co.) are 1·18 and 0·03 respectively. The sulphate content of the food quality carrageenan (28 to 32%) is only slightly reduced following degradation by the above method.

Feeding experiments. The experiments were performed on two groups of 8 adult male albino guinea-pigs (Dunkin-Hartley) 450 to 500 g. The animals were housed separately and fed a vitamin C-enriched cube diet (RGP Dixon & Sons Ltd.). Their drinking fluid was supplied in glass bottles with a ball-valve metal teat.

One group received as drinking fluid over 2 weeks, the 2% degraded carrageenan solution prepared from the food quality carrageenan extract; the second group received over 6 weeks the solution prepared from the crude carrageenan extract. The animals were weighed weekly and were killed by diethyl ether anaesthesia at the end of the feeding experiments.

The large bowel was injected in situ with a 4% solution of formaldehyde in normal saline, carefully removed, and after fixation examined by transmitted light for the presence of ulcerative disease. The number of ulcers in the caecum and colon was counted using a cold light viewer with magnifying lens (10 diopters). The severity of damage in each animal was assessed on an arbitrary 1 to 5 plus scale according to the number of ulcers present (Table 1, footnote).

All of the guinea-pigs in both groups readily accepted the 2% degraded carrageenan solutions as drinking fluid. Nearly all of the animals developed looseness of the stools by the end of the second week, the faeces consisting of soft, moist, poorly formed pellets. This altered bowel function persisted throughout the 6 week period in the second group of animals.

The animals in the group receiving the degraded food quality carrageenan all showed some loss of weight by

Table 1. Incidence and severity of ulceration of the large bowel in guinea-pigs fed 2% degraded carrageenan solutions as drinking fluid.

Drinking fluid (no. of animals)	Length of exp.	Incidence of ulceration of large bowel	No of animals with ulceration of graded severity*				
			1+	2+	3+	4+	5+
1. 2% degraded carrageenan sol from food quality extract (8)	2 weeks	8/8	0	2	2	3	1
2. 2% degraded carrageenan sol from crude extract (8)	6 weeks	8/8	1	5	0	2	0

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

the end of the 2 week period. The animals in the group receiving degraded carrageenan over 6 weeks either failed to gain weight or showed only a slight gain.

The incidence and severity of ulceration in the two groups of animals at the end of the feeding experiments are shown in Table 1. In both groups, there was a 100% incidence of ulcerative disease of the large bowel. The severity of damage was greater in the animals receiving degraded food quality carrageenan than in the animals receiving degraded crude extract carrageenan. In both groups, the severity of damage was greater in the caecum than in the colon.

When fed in the drinking fluid to small laboratory animals, native or undegraded carrageenan derived from the red seaweed *Eucheuma spinosum* will produce ulcerative disease of the colon if supplied in relatively low concentration of 1 to 2% over a long enough period e.g. 2 to 3 months or more. When supplied at high concentrations the solutions are viscous and readily block the metal teats of the drinking bottles. For this reason and also because of the desirability to produce ulcers of the bowel in a shorter time, a degraded carrageenan solution is preferable. Degraded carrageenan extracts, however, are not readily available commercially except in certain countries (e.g. France) where it is used as an anti-peptic agent in the treatment of gastro intestinal disorders. In the U.S., the therapeutic use of degraded carrageenan has been disallowed by the Food and Drugs Administration (1972). Accordingly, an easy method for degrading carrageenan is highly desirable for experimental purposes.

Most methods for the degradation of carrageenan tend to be time-consuming and complicated. Commercial patented methods involve mild acid hydrolysis and relate to the degradation of the powdered carrageenan

extract with the eventual recovery of the degraded product as a dry powder (British Patent 840.623). This is ideal in the manufacture of tablets for therapeutic use, but is a costly procedure for the production of degraded carrageenan in the laboratory. The use of ultraviolet radiation has not been fully investigated and may yet prove to be an effective economical method for degrading carrageenan. The method which we have described above in which the dry native carrageenan powder is exposed briefly to concentrated HCl is both simple and economic, and can be readily carried out in a gastrointestinal research laboratory.

Our experimental results in guinea-pigs have shown that the 2% degraded carrageenan solutions prepared by the above method are readily acceptable as drinking fluid. A limiting factor in the use of concentrations higher than 2% is the salinity of the drinking fluid. The viscosities of the degraded carrageenan solutions are much less than the native carrageenan and no problems with blockage of teats need be anticipated even when using metal teats with ball-valve mechanisms.

These results have also shown that the method of degrading carrageenan does not interfere with the ulcerogenic potential of the algal sulphated polysaccharides. The two degraded carrageenan solutions supplied as drinking fluid to guinea-pigs induced 100% incidence of ulceration of the large bowel within the 2 week and 6 week feeding experiments. Differences in the severity of ulceration probably relate to differences in the purity of the original seaweed extracts.

The use of a 2% degraded food quality carrageenan for the experimental production of ulcerative disease of the large bowel has the advantage of inducing ulceration in a relatively short period i.e. within 2 weeks. This shortening of the pre-ulcerative phase is highly desirable not only for the study of the pathogenic mechanisms involved, but also for the ease of investigation of various factors (including pharmacological and therapeutic agents) which might affect the incidence and severity of ulceration, as well as the rate of healing.

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