parathyroid function(1). The effect of fluoride interaction with bone is primarily in those areas of bone which function to maintain a basic equilibrium between bone and fluid (14). A reduction of this basic level results in a compensatory increase in endogenous parathyroid secretion as indicated by the increased osteoclast counts. If the fluoride concentration of bone is increased sufficiently, the resultant drop in the basic equilibration level is sufficient to produce a secondary effect on the parathyroid-intact animal. This conclusion is further supported by the changes produced by fluoride in removal of hydroxyproline by peritoneal lavage. These changes parallel the calcium changes exactly, indicating that the matrix breakdown, which appears always to accompany the removal of calcium, is similarly affected by the fluoride.

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Antacid and Antiulcer Properties of the Polysaccharide Chitosan in the Rat. (29128)

IRA W. HILLYARD, JOHN DOCZI AND PAUL B. KIERNAN*
(Introduced by Martin M. Winbury)
Departments of Pharmacology and Biochemistry, Warner-Lambert Research Institute, Morris Plains, N. J.

Purified polysaccharides have been evaluated for efficacy in prevention and treatment of peptic ulceration because of their chemical and physical similarity to the mucopolysaccharides of gastric mucin(1). The most efficient (antiulcer) polysaccharide reported thus far has been a sulfated galactosan obtained from sea weed (carrageenin). Chitosan is a glucosamine polymer (poly 2-amino-2-desoxy-β-D glucose) obtained from the chitin of lobsters and crabs. Purified chitosan contains from 7 to 8% nitrogen in the form of titratable primary amino groups. This free amino group content will bind an equivalent amount of acid. *In vitro,* approximately 6 mEq of HCl are bound by 1 g of the compound. The acid binding capacity in combination with the physical properties of a mucopolysaccharide, prompted our evaluation of this material for antiulcer potency. Chitosan however, lacking sulfuric acid ester groups, did not have the antiproteolytic activity inherent in carrageenin(2,3,4,5). Chitosan sulfate was very effective in this respect, but possessed anticoagulant properties. We thus investigated a preparation obtained by coprecipitation of chitosan and Aluminum Hydroxide (Chitosan-AlOH), since it has been shown that aluminates are also significant inhibitors of peptic activity(6,7). This

* Present address: Dept. of Pharmacology, Boston Univ. School of Medicine, Boston.

preparation of chitosan will be shown here to have antipeptic activity in combination with the antacid and demulcent properties of the plain chitosan.

Methods. Adult male rats of the Long-Evans (hooded) strain were fasted 24 hours and subjected to ligation of the pyloric junction under light ether anesthesia(8). Test compounds were administered to each animal at the time of ligation by oral intubation. The desired dose of compound was suspended in distilled water just prior to administration using a volume not exceeding 2 ml per rat. Control animals received the same volume of water. The activity of chitosan and chitosan-AlOH was compared in these tests with carrageenin and with an aluminum hydroxide-magnesium trisilicate antacid mixture (Gelusil†). The latter was used as the tablet granulation and like the polysaccharides was administered as an aqueous suspension. Antacid activity of the compounds was determined from changes in the free gastric acid (HCl) concentration after 4 hours of ligation. At this time, the rats were killed with ether and total gastric contents collected and centrifuged. The fluid volume (less volume of water used to administer the dose) was noted and a 2 ml sample taken for determination of pH and free and total acid concentration. These latter values were measured by potentiometric titration of the sample to pH 3.5 and 7.0, respectively, using 0.02 N NaOH as the titrant. These concentrations were expressed as meq/l. Antipeptic potency was estimated from changes occurring in the proteolytic activity of the 4 hour secretions after conversion to pH 1.2. This activity was determined using a modification of the Anson hemoglobin digestion method(9), and expressed as hemoglobin units $\times 10^4$ per ml of gastric juice.

Antiulcer activity was observed as a reduction in the arbitrarily assessed severity of ruminal ulceration occurring in rats during an 18-hour period of ligation. Ulcer severity was numerically scored as follows: 0 = no irritation or ulceration; stomach normal in appearance; 1 = gastric irritation and erythemia, but no ulceration; 2 = minor ulceration with several small ulcers observed on close examination; 3 = several large ulcers or numerous small lesions; 4 = severe ulceration with perforation.

Gastric demulcent activity was evaluated in dogs as well as rats. In both cases, the ability of these compounds to protect the stomach from the irritation and mucosal damage produced by a large oral dose of aspirin was used as a test for this activity. In rats, 0.5 g of aspirin was given to each animal as an aqueous suspension at the time of pylorus ligation. After 5 hours, the animals were sacrificed and degree of mucosal damage assessed using the numerical scoring system described above. In dogs, 2 g of powdered aspirin was given as a single dose in gelatin capsules. These dogs were carefully selected and maintained free from fecal occult blood for at least 1 week prior to administration of aspirin or aspirin-demulcent combination. Each day, for 7 days following administration of aspirin, the morning feces was examined for the presence of occult blood and quantitated using Hams' benzidine filter paper test(10). Serum salicylic acid level was determined in each animal at 30, 60 and 120 minutes after aspirin administration to establish whether a decrease in occult blood was due to a protective effect of the agent on the gastric mucosa or was the result of a physical or chemical binding of the aspirin by the agent *in vivo*. Serum salicylic acid was determined using the ferric ion salicylate colorimetric reaction after extraction in ethylene dichloride(11).

Results. The comparative antacid activity is given in Table I. At the 500 mg/kg dose level, both chitosan and chitosan-AlOH reduced the control 4 hour free acid concentration by the same amount—39.6 meq/l. This value is somewhat lower than the $\geq$50 meq/l reduction predicted from *in vitro* analysis. However, this value is still some 3 times greater than the amount of acid neutralized by the same dose of Gelusil. Carrageenin neutralized essentially the same amount of free acid as the chitosans with the 500 mg/kg dose. None of the agents tested

† Trade name, Warner-Chilcott Laboratories.

TABLE I. Comparative Antacid Effect in 4-hour Pylorus Ligated Rats.

Agent	Oral dose (mg/kg)	No. of rats	4-hr vol (ml/rat)	pH	Mean gastric acidity (mEq/l ± S.E.) Free acid	Total acid
Water		114	7.3 ± .2	1.20 ± .01	92.6 ± 1.6	106.9 ± 1.5
Chitosan	500	10	9.0 ± 1.0	1.48 ± .10	53.0 ± 8.3	102.8 ± 3.5
	1000	12	9.7 ± .3	1.68 ± .10	30.3 ± 3.7	97.2 ± 3.4
Chitosan-AlOH	250	10	10.3 ± .6	1.28 ± .02	81.4 ± 4.3	111.0 ± 2.1
	500	12	7.4 ± .4	1.38 ± .06	53.0 ± 4.1	102.0 ± 2.7
Gelusil	500	10	9.0 ± 1.0	1.36 ± .10	80.4 ± 9.9	117.4 ± 8.8
	1000	10	7.7 ± 1.0	1.92 ± .20	39.4 ± 8.3	75.4 ± 6.9
Carrageenin	250	5	9.1 ± .8	1.32 ± .02	88.8 ± 2.9	99.2 ± 3.0
	500	5	7.2 ± .9	1.52 ± .20	54.2 ± 10.9	72.2 ± 10.1

TABLE II. Antipeptic Evaluation. The proteolytic activity of gastric juice collected from rats 4 hours after pylorus ligation and drug administration.

Agent	Oral dose (mg/kg)	No. of rats	Peptic activity units* (19/20 conf limit)	Mean decrease in peptic-activity (%)
Water		15	8.42 (6.97- 9.87)	
Chitosan	500	5	9.10 (6.76-11.44)	0 (+ 8.2)
	1000	6	10.90 (9.26-12.50)	0 (+29.5)
Chitosan-AlOH	125	5	5.90 (.81-11.00)	30.0
	250	5	1.80 (0 - 7.03)	78.6
	500	6	1.23 (0 - 6.58)	85.4
Carrageenin	250	5	2.12 (.55- 3.69)	74.8
	500	5	1.50 (.47- 2.53)	82.2
Gelusil	500	5	7.80 (5.23-10.37)	7.4
	1000	5	2.90 (0 - 5.98)	65.6

* Hemoglobin units × 10^4 per ml of gastric juice determined after conversion of pH to 1.2.

decrease the secretory volume of these animals, but did, in some cases, increase this value. Total gastric acid was not decreased by chitosan or chitosan-AlOH, but higher doses of Gelusil and carrageenin did produce a moderate reduction.

The effect on the proteolytic activity of 4 hour secretions is listed in Table II. Plain chitosan increased the mean proteolytic activity of these secretions but chitosan AlOH produced a definite inhibition of activity with doses as low as 125 mg/kg. The average antipeptic activity of the coprecipitate was similar to that observed with equal doses (250 and 500 mg/kg) of carrageenin, but was much more variable than the latter. The highest dose (1000 mg/kg) of Gelusil also produced a significant decrease in peptic activity.

Chitosan and chitosan-AlOH both had a definite gastric demulcent effect in both rats and dogs. In rats, a significant reduction in mucosal erosion was affected by a dose of 1 g/kg of chitosan and 0.5 g/kg of chitosan-AlOH (Fig. 1). The demulcent activity of these doses of chitosan was equiactive with 1 g/kg of carrageenin and a 0.5 g/kg dose of Gelusil. In dogs, 2 g of either chitosan or chitosan-AlOH reduced both the duration and degree of gastrointestinal bleeding induced by the 2 g dose of aspirin (Table III). This protective effect was not accompanied by any alteration in either rate of aspirin absorption or level of serum salicylic acid 1 and 2 hours after aspirin ingestion. The effects of carrageenin and Gelusil were not evaluated in this test.

The effect of chitosan on the peptic ulceration occurring in rats during an 18 hour period of ligation was characterized only by a reduction in ulcer severity (Table IV). The number of animals dying of ulcer perforation was reduced and the mean ulcer severity score decreased, but the incidence of ulceration was not altered. The reduction in ulcer severity was not remarkable with doses

TABLE III. Gastric Demulcent Activity in Dogs. Effect on gastrointestinal bleeding induced by a single oral dose of aspirin.

Test compound (dose/dog)	Gastrointestinal bleeding: Duration, days	Fecal occult blood score* ± S.E.	Serum salicylic acid mg % ± S.E.†
Aspirin alone (2 g)	7	2.43 ± .20	38.8 ± 3.0
Chitosan‡ (2 g)	4	1.86 ± .44	40.5 ± 3.0
Chitosan-AlOH‡ (2 g)	3	1.67 ± .43	44.3 ± 5.9

* Avg maximal score of 7 dogs. The same animals were used for both control (aspirin alone) and for test (aspirin + chitosan) evaluations.
† Concentration 2 hr after aspirin ingestion.
‡ Administered in gelatin capsules as dry powders in combination with aspirin.

less than 500 mg/kg. Chitosan-AlOH was no more effective than plain chitosan despite the antipeptic activity of the former. A 500 mg/kg dose of carrageenin completely prevented ulceration in all animals tested. Gelusil was not significantly effective in this test, at least with the doses used.

Discussion. The ulcerigenic procedure used in this investigation is characterized by the accumulation, in the ligated stomach, of a large quantity of highly acid gastric juice with strong proteolytic activity(8). The ulceration observed is not located in the mucosal portion of the rat stomach, but is found in the relatively unprotected ruminal area. Apart from secretory inhibition, a decrease in this peptic ulceration could be due to either a demulcent protection of this portion of the stomach or to a decrease in the free acid concentration. Ulceration could also be prevented by inhibiting peptic activity separately. The antiulcer effect of chitosan is probably due to a combination of both physical protection and acid neutralization. The antacid effectiveness of chitosan during a 4-hour interval is approximately 4 meq/g.

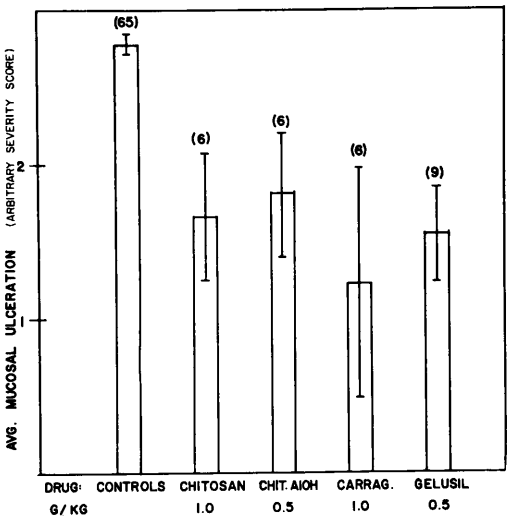

FIG. 1. Gastric demulcent activity in rats. Protection against aspirin (0.5 g) induced mucosal ulceration in 5-hr pylorus ligated animals. Center vertical line in each bar is standard error of avg ulcer severity. Number in parentheses indicates number of animals tested.

This acid binding capacity is moderate compared to fully reactive alkali antacids, but is sufficient to afford an effective decrease in hydrolytic activity of the secretions. The antacid potency of chitosan is, in fact, 3 to 4 times greater than an equivalent amount

TABLE IV. Antiulcer Activity. Ulcer protection afforded rats during 18 hours of pylorus ligation.

Agent	Oral dose (mg/kg)	No. of rats	No. of rats with ulcer (mortality*)	Ruminal ulcer severity Numerical score† (19/20 confid limit)
Water		24	24 (22)	3.25 (2.82-3.68)
Chitosan	500	12	12 (10)	1.83 (.60-3.06)
	1000	10	8 (0)	1.40 (0 -3.46)
Chitosan-AlOH	125	10	10 (9)	2.80 (1.79-3.83)
	250	10	10 (8)	2.40 (1.29-3.51)
	500	10	10 (4)	1.80 (.13-3.47)
Carrageenin	250	12	12 (1)	2.83 (1.72-3.94)
	500	12	0	0 (1.72-3.94)
Gelusil	500	10	8 (6)	2.60 (.18-4.00)
	800	12	12 (6)	2.42 (.70-4.00)

* Number of animals dying of ulcer perforation.
† Avg value. Maximal severity score = 4.

of partially reactive alkali antacid, Gelusil, or of purified gastric mucin(12). The demulcent potency of chitosan is reflected by a marked mucosal protection observed in both rats and dogs against irritating doses of aspirin.

Chitosan-AlOH, in addition to having the antacid and demulcent potency of plain chitosan was also an inhibitor of peptic activity. However, chitosan-AlOH was no more effective than chitosan in preventing peptic ulceration nor was the coprecipitate as protective as carrageenin despite our observation that both compounds produced the same average decrease in peptic activity. The more significant antiulcer activity of carrageenin may be due to a more efficient mechanism of proteolytic inhibition. Aluminates inactivate pepsin directly, probably by colloidal adsorption(7). Carrageenin, on the other hand, does not react directly with pepsin, but combines strongly with protein substrate hydrolyzed by pepsin(3,5). Further, the protein-carrageenin complex formed is additionally protected from peptic digestion by the polysaccharide. This reaction of carrageenin with protein effectively produces a demulcent mass over the total surface of the stomach and, especially, in and around an ulcer. A total inactivation of pepsin by aluminates would thus have to be accomplished in order to obtain the same degree of ulcer protection as that produced by the substrate protection affected by the sulfated polysaccharides.

Conclusions. Chitosan, a polymer glucosamine obtained from the chitin of lobsters and crabs, affords a moderate, but definite protection to pylorus ligated rats against peptic ulceration of the rumin. This antiulcer activity has been shown to be due to a moderate capacity to bind free gastric acid and to a significant ability to act as a demulcent. Gastric demulcent activity was also observed in unligated dogs given a large dose of aspirin. Antipeptic activity was not observed with chitosan, but was with an aluminum hydroxide coprecipitate of chitosan. This latter material was almost equal to carrageenin in inhibiting proteolytic activity of gastric secretions but was not as effective as the sulfated galactosan in preventing peptic ulceration. A possible explanation of the lesser antiulcer potency of chitosan-AlOH is discussed.

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