THE EFFECT OF A SULPHATED POLYSACCHARIDE UPON THE DIFFUSION OF PEPSIN THROUGH MUCIN

By W. Anderson

From the Evans Medical Research Laboratories, Liverpool, 24

Received May 18, 1961

Degraded carrageenan, a sulphated polysaccharide, is shown to hinder the diffusion of pepsin through mucin *in vitro* when it is mixed with the mucin before the application of the pepsin solution. It is suggested that this property is part of the mechanism by which degraded carrageenan protects experimental animals from histamine ulceration.

Experimental animals can be protected from histamine-induced peptic ulceration by giving them certain sulphated polysaccharides orally (Levey and Sheinfeld, 1954; Anderson and Watt, 1959). Sulphated polysaccharides diminish peptic activity *in vitro* mainly by reacting with and protecting the substrate, rather than by inactivating the enzyme (Anderson, 1961). Their mode of action *in vivo* is unlikely to be by direct antipeptic activity. A partial explanation may be that the polysaccharides protect the mucosa from the gastric juice by reacting with mucoprotein to give an increase in strength of its mucinous properties, thereby causing an increased resistance to the diffusion of gastric secretion through the mucoprotein to the mucosa. The work now reported was designed to demonstrate this hypothesis *in vitro*.

Experimental

Materials and Methods

Sulphated polysaccharide. Degraded carrageenan* was used (Anderson, 1961). A 10 per cent solution gave a pH of 6·8.

Mucin. 10 g. gastric mucin (Armour Laboratories) intimately mixed with 100 ml. water, was centrifuged to deposit gross insoluble matter, and the slightly opalescent viscous supernatant solution was used. This was called 10 per cent mucin; it contained 0·4 per cent nitrogen and had a pH of about 5·5. In the experiments it was diluted either with equal parts of water to give 5 per cent mucin, or with equal parts of a 10 per cent aqueous solution of degraded carrageenan to give 5 per cent mucin containing 5 per cent degraded carrageenan. The pH of the mucin and of the degraded carrageenan solutions were not adjusted, except where indicated.

Pepsin solution. Granular 1:10,000 pepsin at a concentration of 20 mg./ml., adjusted to pH 1·6 with HCl.

Acid solution. Solution of HCl in water at pH 1·6.

Haemoglobin solution. 0·5 per cent bovine haemoglobin enzyme substrate powder (Armour Laboratories) in HCl solution adjusted to pH 1·6.

Method. A double-end cell was used to simulate idealised conditions in the gastrointestinal tract.

*Ebimar (Evans Medical Ltd.).

The cell was constructed from a tube which was divided in two by a porosity 4 fritted disc 10 mm. in diameter and 2 mm. thick, which was fused into the centre of the tube. The ends of the tube were of a length such that when the rubber stoppers were inserted to a given mark the volume in each limb was 5 ml. The size disc used was found to be the most suitable because it allowed reasonable diffusion rates without applying pressure, while not allowing bulk streaming of the liquids. With simple diffusion experiments using pepsin solution and discs of the same nominal porosity, it was found that each tube had a specific diffusion rate, which was related approximately to the suction-filtration rate. Tubes having the same resistance to flow characteristics were grouped in threes. Each group of cells was used in turn for each experiment (that is: no mucin,

TABLE I

THE DIGESTION (INDICATED BY OPTICAL DENSITY × 100) OF HAEMOGLOBIN IN 6 HR. BY PEPSIN IN THE ABSENCE OF MUCIN; AFTER DIFFUSION THROUGH MUCIN; AND AFTER DIFFUSION THROUGH MUCIN PLUS DEGRADED CARRAGEENAN

Cell number	No mucin	Mucin	Mucin plus degraded carrageenan
1	46·5	7·5	10·0
5	38·0	8·5	8·0
6	32·5	5·0	9·5
17	47·5	16·5	4·5
8	42·0	27·0	8·5
9	56·5	29·5	8·0
2	37·5	12·0	7·0
3	21·0	24·0	12·0
13	84·0	19·5	18·0

Significance of difference between the means of columns two and three taken alone: $t = 2{\cdot}19$; $P = 0{\cdot}02$–$0{\cdot}05$

mucin, mucin plus degraded carrageenan). Before use, and after cleaning, the tubes were washed with acid solution by suction, the disc being left saturated with acid solution. The inner walls of the tube limbs were dried and 5 ml. of haemoglobin solution at 35° placed in the lower limb. A hypodermic needle inserted through the rubber stopper allowed it to be inserted to the mark without including air and without forcing the solution into the fritted disc. The needle was then removed. Into the upper limb was placed: 0·5 ml. of 5 per cent mucin; or 0·5 ml. of 5 per cent mucin containing 5 per cent degraded carrageenan; or 0·5 ml. water, to give a layer 7 mm. deep. The pepsin solution was layered on to this so that, apart from the water samples, a distinct interface was formed. All solutions were at 35°. After filling, the tubes were stoppered and supported vertically in a water bath controlled at 35°. After 6 hr. incubation the haemoglobin solution was poured into 10 ml. of 10 per cent trichloroacetic acid. The filtrate from this was taken to measure the amount of peptic digestion using Folin-Ciocalteu's reagent as described by Anderson (1961); the optical densities of the solutions indicating the extent of peptic digestion of the haemoglobin in the lower limb, this in turn being a measure of the pepsin gaining access to, and being incubated with, the substrate within 6 hr.

Results

The results are given in Table I.

Effect of the Sulphated Polysaccharide in the Pepsin Solution

Similar experiments were made where different amounts of degraded carrageenan were dissolved in the pepsin solution. More than 1 per cent of degraded carrageenan gave rise to difficulty in layering the solution onto the mucin because of alteration in density. Nevertheless, even 1 per cent degraded carrageenan in the pepsin layer did not retard diffusion of pepsin through the mucin under these conditions, as compared with the diffusion when degraded carrageenan was absent from the pepsin solution. The results of the mean digestions (indicated by optical density $\times$ 100) of haemoglobin in 6 hr. are: by pepsin in the absence of mucin, 49·8; after diffusion through mucin, 17·8; and after diffusion through mucin, the pepsin first having been mixed with degraded carrageenan, 20·7.

Diffusion of Carrageenan

Some degraded carrageenan diffused from the mucin through the fritted disc, but the quantity diffusing in 6 hr. was small (ca. 0·2 mg.) and would not be expected to account for the diminished digestion seen. This experiment was made by replacing the haemoglobin solution with acid solution and estimating the degraded carrageenan found therein after the 6 hr., with toluidine blue. The results are: mg. degraded carrageenan found in the lower limb in 1 hr. 0; 3 hr. 0·04; 5 hr. 0·2; 6 hr. 0·2.

Diffusion of Acid

The diffusion of acid from the pepsin layer through the mucin could be seen as a descending opacity due to precipitation in the mucin layer and this was usually complete in the first half hour. When degraded carrageenan was present in the mucin the acid did not do this to the same extent but a thin (about 1–2 mm.) opaque pellicle formed at the interface. This began to form as soon as the acid or acid-pepsin was layered and appeared to impart a cohesiveness to the mucin surface which was stronger as a result, and obviously withstood the occasional impact during layering much better than when degraded carrageenan was absent.

Depth of Mucin Layer

Layers of 0·2 ml. (giving 3 mm.) and 1 ml. (giving 13 mm.) mucin were also tried. 0·2 ml. gave a layer so shallow that floating of the pepsin solution was a hazardous and tiresome operation, whereas the 1 ml. layer gave results similar to those with 0·5 ml.

Discussion

The results show that a sulphated polysaccharide added to mucin hinders the passage of pepsin through the mucin. They are comparative and the conditions of the experiment are admittedly ideal, a steadier state being substituted for the dynamic state existing in natural conditions.

The concentrations and quantities of materials have been chosen for convenience and only in their disposition do they resemble the natural order inasmuch as the acid gastric juice bathes a mucinous layer which is supported on closely-knit structures which separate it from the mucosa. The haemoglobin solution is a convenient experimental substitute for denatured digestible tissue. The mucin was used without adjustment of pH which was 5·5 and therefore probably close to its natural reaction. It was thought that if degraded carrageenan was mixed with it at this pH, reaction between them would mostly occur as the acid and, more slowly, the pepsin, diffused through the layer giving a greater opportunity for impeding the progress of the pepsin.

The concentrations of the mucin, pepsin and degraded carrageenan were chosen principally on a density basis to avoid mixing or inversion. Similarly in the experiments where the sulphated polysaccharide was present in the pepsin layer its concentration could not have been increased greatly without causing the same difficulty. The depth of the mucin layer was also of importance, and this is believed to be a natural factor in its protective function. It was observed that layering of the pepsin solution without deformation of the interface was markedly easier on mucin containing sulphated polysaccharide, and this was thought to be due to the rapid formation on the surface of the mucin of a "pellicle" which could clearly be seen when it expanded to about 1–2 mm. in depth during the experiment.

The difference between the effect of mucin and that of mucin plus degraded carrageenan can be exaggerated by prolonging the incubation period. Although periods longer than 6 hr. become unrealistic, it was clear in these experiments that the passage of pepsin through, and from, mucin containing sulphated polysaccharide is very severely curtailed.

This process of restricting the access of pepsin from mucin to the mucosa is suggested as a contributory factor in the ulcer-preventing activity of sulphated polysaccharides which has been reported in the laboratory animal. It is now known that these substances also diminish peptic activity by protecting the substrate or digestible tissue in the presence of acid. It is not intended to imply that these two experimental observations explain the dramatic protection of the experimental animals from the ulcerative effects of large volumes of strong gastric juice secreted under histamine or stress stimulation, but they must be aspects of a many-sided action on the elements of gastric secretion.

References

Anderson, W. (1961). *J. Pharm. Pharmacol.*, **13**, 139–147.
Anderson, W. and Watt, J. (1959). *J. Physiol.*, **147**, 52–53P.
Levey, S. and Sheinfeld, S. (1954. *Gastroenterology*, **27**, 625–628.

The paper was presented by THE AUTHOR.