Properties of Gastric and Duodenal Mucus: Effect of Proteolysis, Disulfide Reduction, Bile, Acid, Ethanol, and Hypertonicity on Mucus Gel Structure

A. E. BELL, L. A. SELLERS, A. ALLEN, W. J. CUNLIFFE, E. R. MORRIS, and S. B. ROSS-MURPHY

Department of Physiological Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne; National Institute for Medical Research, Mill Hill, London; Unilever Research, Colworth Laboratories, Sharnbrook, Bedford, United Kingdom

Small deformation oscillatory rheologic measurements have been used to investigate the structure of human and pig gastric mucus and pig duodenal mucus. All three secretions had viscoelastic properties characteristic of water-insoluble, viscoelastic gels. Mucus will flow and anneal if damaged, due to the making and breaking of its elastic structure, the measured lifetime of which was 10–120 min. Mucus reconstituted by concentration of the purified glycoprotein (pig gastric and duodenal mucus) had the same viscoelastic properties as the fresh mucus, giving evidence that the glycoprotein alone will reproduce the rheologic characteristics of the mucus. The structure of fresh mucus gel was unaffected by prolonged exposure to the following mucosal damaging agents: undiluted pig bile, 20 mM sodium taurocholate or 20 mM sodium glycocholate (all at pH 2, 6, and 8), HCl at pH 1, 2 M NaCl, and ethanol <40% (vol/vol). Higher concentrations of ethanol, >40% (vol/vol), caused dehydration and denaturation of mucus. Proteolysis by pepsin and other enzymes resulted in solubilization of the mucus gel with a complete change in the properties from an "elastic" gel to those of a "viscous" liquid. A similar collapse of mucus gel structure was observed after reduction of disulfide bonds in 0.2 M mercaptoethanol, but only after incubation for at least 50 min. This study demonstrates the stability of mucus to several mucosal damaging agents. It is proposed in vivo that although adherent gastroduodenal mucus allows penetration of these agents to the underlying mucosa, it can remain in situ and continue to protect against acid (with HCO_3^-) and pepsin, thus minimizing mucosal damage and maximizing repair.

Gastroduodenal mucus is secreted as a water-insoluble gel adherent to the mucosal surface and is also present as a viscous, soluble form in the lumen. The adherent gel covering the mucosal surface is considered to have a protective role against (a) acid by acting as a stable mixing barrier (with the epithelial HCO_3^- secretion) (1–4), (b) luminal pepsin by forming a diffusion barrier (5,6), and (c) mechanical damage by acting, together with soluble mucus, as a lubricant (7). By direct examination of ex vivo sections of rat stomach and duodenum the adherent mucus gel is observed to be a continuous layer of variable thickness, 5–200 μm and occasionally 400 μm (8). Values for human stomach were about twofold greater. Mucus thickness (mean 73 and 82 μm in the rat stomach and duodenum, respectively) was essentially unchanged after topical application, intragastrically, of the following mucosal damaging agents: 60%–100% (vol/vol) ethanol, 80 mM sodium taurocholate, indomethacin 30 mg/kg, 0.6 M HCl, or 2 M NaCl (9,10). These studies emphasize the relative stability of the adherent mucus in vivo but, for a full understanding it is necessary to correlate this with the structure of the gel.

Much information on mucus structure has come from studies on the isolated glycoproteins themselves. In pig gastric mucus the gel-forming properties are reproduced by a purified glycoprotein of relative molecular mass (M_r) $\sim 2 \times 10^6$ (11). In dilute solution this mucus glycoprotein has a high viscosity and occupies a large volume (12). As the concentration of mucus glycoprotein is increased, the vis-

Received April 2, 1984. Accepted July 23, 1984.

Address requests for reprints to: Adrian Allen, Department of Physiological Sciences, Medical School, Framlington Place, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH, United Kingdom.

0016-5085/85/$3.30

cosity of the solution rises steeply and at a concentration above ~5% by weight, a reconstituted mucus gel is formed. This gastric mucus glycoprotein is a covalent polymer of subunits (M_r ~5×10^5) joined together by disulfide bridges (6,13). These intersubunit disulfide bridges are located in the nonglycosylated parts of the protein core and it is these regions that are susceptible to proteolytic enzymes (14). Thus proteolysis or reduction cleaves the gastric mucus glycoprotein into the subunits of markedly lower viscosity, with loss of the gel-forming properties of the glycoprotein and solubilization of the mucus gel. Although a high viscosity in dilute solution is clearly an indication of gel-forming potential in mucus, such viscosity studies can be related only indirectly to the structure of the gel itself. This is because the rotational shear used in viscosity measurements will disrupt mucus gel structure and also because interactions not seen in dilute solution can take place at the high concentration of glycoprotein present in the gel matrix.

By means of mechanical spectroscopy, information can be obtained about mucus gel structure without its disruption (15). In this technique mucus gel is sandwiched between a flat plate and shallow cone and subjected to small deformations at different oscillatory frequencies. From the magnitude and phase of the stress generated in resistance to the applied deformation, the storage modulus G′, the elastic or solid component, and the loss modulus G″, the viscous or liquid component, can be calculated. Previous rheologic studies have shown that human respiratory mucus behaves as a weak viscoelastic gel (16–18). Similar rheologic properties were observed for pig gastric mucus although it has less tendency to flow than respiratory mucus (11). A model has been proposed for gastric mucus structure in which the functional interactions between the native polymeric glycoprotein molecules arise noncovalently by interdigitation of the carbohydrate chains (19).

Here we have used mechanical spectroscopy to study the properties of the mucus gel and the effect on its structure of proteolysis, reduction of disulfide bonds, and the mucosal damaging agents acid, bile, ethanol, and hypertonic saline that have been implicated in breakdown of the gastroduodenal mucosal barrier (20,21). In these studies we have investigated gastric and duodenal mucus from the pig animal model, together with mucus from resected human stomachs of peptic ulcer patients.

Materials and Methods

Pig gastric and duodenal mucus gel samples were obtained by gently scraping the mucus gel from the surface of the washed mucosa with a small plastic scoop or a glass slide. Human gastric mucus samples were obtained after surgical resection of the antrum from gastric ulcer patients. Some fresh mucus samples were investigated as soon as possible (1 h) after collection but most were immediately frozen and stored at −20°C until required. The glycoprotein content of the native gels was assayed by the modified periodic acid-Schiff method (22) after the sample of gel had first been exhaustively digested with papain and dialyzed against saline to remove protein.

Reconstituted mucus gels were formed from glycoprotein isolated from the native pig gastric or duodenal mucus. Pooled mucus scrapings from 15–25 stomachs or 30–40 duodenums were diluted 1:5 with 0.2 M NaCl/0.02% sodium azide solution and homogenized at 4°C. After centrifugation (9000 rpm) at 4°C for 1 h the supernatant was separated from the cell debris and strained through glass wool to remove lipid material. The separation of glycoprotein from free protein and nucleic acid was by equilibrium centrifugation in a CsCl density gradient (23,24). Mucus glycoprotein samples were further purified from these contaminants in a second subsequent CsCl density gradient. The glycoprotein fractions after centrifugation were pooled and dialyzed against 0.2 M NaCl/0.02% azide solution and concentrated by vacuum dialysis. The isolated glycoprotein was concentrated (pig gastric ~50 mg/ml, pig duodenal ~40 mg/ml) to give reconstituted gels with glycoprotein concentrations comparable to that of native gels.

Dynamic oscillatory measurements on native gels or gels reconstituted from isolated glycoproteins were performed on a Rheometrics mechanical spectrometer (Rheometrics, Springfield, N.J.), using a cone and plate system (cone diameter 25 mm, cone angle 0.1 radian). Sinusoidal deformation of the sample under test (sample volume ~1.5 ml) was produced by an electronically controlled servo system (range 10^{-3}–10^2 rad/s, with a resolution of better than 0.1% per decade) driving the cone. The energy transmitted to the lower plate was detected by a transducer (Rheometrics, type ST, 0.01–10 g/cm) mounted on an air bearing to reduce frictional losses. Environmental control of the sample chamber was forced by air convection, the air being supplied from a thermostatted water bath and humidified at the working temperature of 25°C. Sample temperature was regulated to ±0.30°C and sensed using a platinum resistance thermometer. The storage G' (elastic component) and loss G'' (viscous component) and the complex modulus were measured as functions of both strain (γ) and frequency (ω) (strain range 5%–100%, frequency range 10^{-2}–10^2 rad/s).

After loading, all samples were allowed to equilibrate (15 min) to the measuring temperature and to relax from any stresses that may have been induced by the loading procedure. The mechanical properties of the mucus samples were unchanged over a period of 2.5 h (four successive frequency scans), showing that dehydration of the gel or other mechanical damage was not a factor in the measurements.

Mechanical spectra of native mucus were measured on gel samples taken from single stomachs (pig n = 15; human n = 2) and duodenum (pig n = 8). For all

treatments with mucosal damaging agents, samples (1.5 ml) were taken from pooled batches of mucus. To examine the effects of high salt or varying pH on the mechanical properties, the gels were dialyzed at 4°C for 24 h against one of the following: 0.2 M NaCl, 2.0 M NaCl, distilled water, 0.2 M disodium hydrogen phosphate/sodium dihydrogen phosphate at pH 8.0 and 7.0, 0.2 M sodium acetate/acetic acid at pH 5.0, 0.2 M glycine/HCl at pH 2.0, or 0.2 M NaCl adjusted to pH 1.0 with HCl; all solutions included 0.02% azide except for distilled water.

The effects of pig gallbladder bile were examined using two protocols. First, gel samples were submerged in twice their own volume of a solution of bile or a given bile salt and gently agitated at 25°C for 24 h. The bile preparations were poured off from the gel after this interval and the gel was tested by rheogoniometry. In separate experiments the mucus was dialyzed against bile or a given bile salt preparation for 18 h at 4°C. The following bile salt solutions were investigated: fresh bile from the pig gallbladder, 20 mM sodium taurocholate, and 20 mM sodium glycocholate. Each of these preparations was adjusted to the following pH values: pH 8 and pH 6 (0.2 M disodium hydrogen phosphate/sodium dihydrogen phosphate) and pH 2 (0.2 M KCl/HCl). All buffers included 0.02% azide. The effect of fresh bile without added buffer was also investigated (pH 6).

The effect of ethanol on the rheologic properties of the mucus gel was examined after exposure for 1.5 h at 37°C to 10%, 20%, 30%, 40%, 50%, or 60% ethanol (final concentration of ethanol). The mucus in this instance was submerged in a volume of ethanol solution that was four times the volume of the gel.

Mucus was reduced by dialyzing for 24 h at 4°C against 0.2 M mercaptoethanol in 0.2 M disodium hydrogen phosphate/sodium dihydrogen phosphate buffer at pH 8.0 (0.02% azide). Reduction of mucus samples was also performed at 37°C for varying times. The mucus samples were gently agitated in four times their volume of reducing buffer. The final concentration of mucus and buffer was 0.2 M with respect to mercaptoethanol and disodium hydrogen phosphate/sodium dihydrogen phosphate buffer at pH 8.0.

Proteolytic digests of the gel samples were obtained using pepsin (1 mg/ml) or papain (20 μg/ml) at 37°C (14). The pig gels were submerged in pepsin buffer (0.2 M citric acid adjusted to pH 2.2 with 5 M NaOH) and agitated for varying time intervals. Digestion by papain was with both the mucus and enzyme contained in a dialysis sac immersed in 0.1 M potassium dihydrogen phosphate/disodium hydrogen phosphate buffer at pH 6.5 containing 0.002 M cysteine/0.005 M ethylenediaminetetraacetic acid. The volume of the buffer used was four times the volume of the gel.

After measurement of their mechanical spectra, all gel samples were stored at −20°C until analyzed. The glycoprotein and protein concentrations within each sample were assayed by the periodic acid-Schiff method (22) and the Lowry method (25), respectively. All mucus samples were also analyzed for the presence of degraded glycoprotein by gel filtration on a Sepharose 2B column (Pharmacia Ltd., Piscataway, N.J.) after homogenization to solubilize the mucus. The glycoprotein was eluted with 0.2 M NaCl/0.02% azide in all cases and the fractions were assayed by both the periodic acid-Schiff method for glycoprotein and the Lowry assay for protein.

Results

Mechanical Properties of Native Pig Gastric Mucus

Native mucus had the appearance of a sloppy, thixotropic gel that would slowly flow, and anneal if sectioned. The values of the storage, G' (elastic), and loss, G'' (viscous), moduli were measured for 15 preparations of native gel taken directly from the surface of different pig stomachs. The pattern of variation of the storage and loss modulus over the frequency range (10^{-2}–10^{2} rad/s) and strain (5%–100%) level was always characteristically the same. Throughout the frequency range accessed, the storage modulus was substantially higher than the loss modulus and both parameters showed little frequency dependence (Figure 1). At the prevailing concentration such behavior is more characteristic of a true gel network than a solution of "entangled" polymer chains (15,26). There was a <10% fall in the values of the storage and loss moduli when strain was increased from 5% to 100% (at a fixed frequency of 10 rad/s; not shown), and at no point was there a sudden decrease that would indicate rupture of the gel structure. Repetition of frequency and strain scans on the same gel sample after 45 and 15 min,

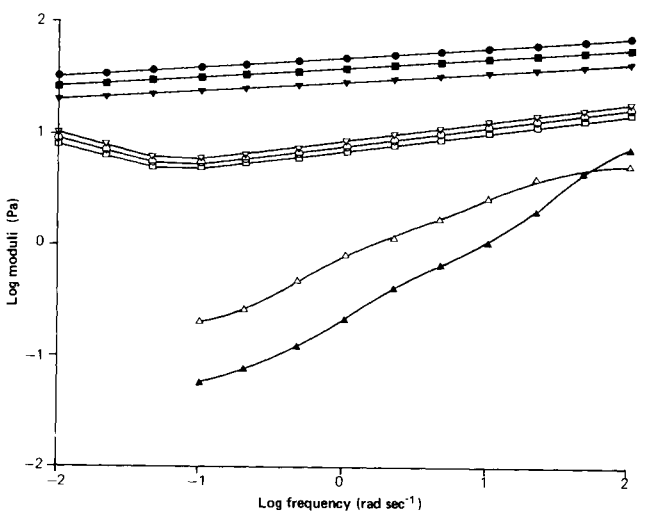

Figure 1. Mechanical properties of pig gastric mucus: plots of the storage modulus (*closed symbols*) and loss modulus (*open symbols*) against frequency for a typical sample of fresh mucus (●○), mucus reconstituted by concentration of purified glycoprotein (50 mg/ml) (■□), mucus incubated for 24 h at 25°C with bile from the pig gallbladder (▼▽), or mucus incubated for 10 h at 37°C with pepsin (1 mg/ml) in 0.2 M citric acid adjusted to pH 2.2 with 5 M NaOH (▲△).

respectively, gave identical results to those originally obtained, showing that the mucus gel could recover and was not permanently changed by the mechanical distortion of the measurement process.

It is the pattern of behavior of the storage and loss moduli, rather than their absolute values, that define gel structure and this was the same for all 15 individual samples of pig gastric mucus. These parameters showed only a small dependence on frequency and the storage modulus was substantially higher than the loss modulus throughout. For native pig gastric mucus the absolute values of the storage (20–48 Pa) and loss (4.0–7.6 Pa) moduli varied from sample to sample. A direct comparison of the ratio of "gellike" and "liquidlike" behavior characterized by these moduli may be established by the ratio of the loss to the storage moduli, $G''/G' \equiv \tan \delta$; $\tan \delta > 1$ indicates a predominantly "viscous" response (liquidlike), whereas $\tan \delta < 1$ indicates an "elastic" response (gellike). Observed values of $\tan \delta$ for pig gastric mucus were all in the range of 0.15–0.35 throughout the frequency range accessed. For calculation of $\tan \delta$ for different samples, values of the storage and loss moduli at a frequency of 1 rad/s were taken. This was the lowest frequency at which the moduli were obtainable for all samples studied, in particular the proteolytically digested and reduced mucus samples.

The total concentration of glycoprotein in native gastric mucus varies from 35 to 49 mg/ml with a mean of 47 mg/ml, whereas the protein content ranges from 12.3 to 24.7 mg/ml with a mean of 17.4 mg/ml ($n = 15$). Although the protein estimation is crude, it does show the presence of some protein in the glycoprotein gel, although the mucus glycoprotein molecule contains only 13% (wt/wt) protein (14). It is not known how much of this protein arises from cellular material removed during the collection procedure or comes from the mucus secretion.

Mucus samples taken from the body (in pig equivalent to the fundic and cardiac regions) of six separate stomachs were compared with material from the pylorus of the same stomachs. There was no difference in the pattern of variation in the moduli as a function of either frequency or strain between the two regions.

Mechanical Properties of Human Gastric Mucus and Pig Duodenal Mucus

Two samples of human antral mucus, both from patients with gastric ulcer, were scraped from nonulcerated areas of the mucosa and the rheologic properties were studied. The mechanical spectra of human gastric mucus was the same as that described previously for pig gastric mucus (Figure 2). There was minimal change in the moduli with increasing

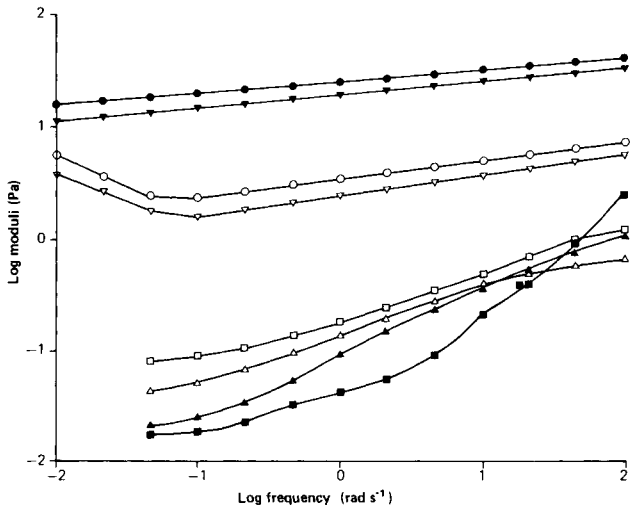

Figure 2. Mechanical properties of human gastric mucus: plots of the storage modulus (*closed symbols*) and loss modulus (*open symbols*) against frequency for a typical sample of human mucus (●○), human mucus dialyzed for 24 h at 4°C against 0.2 M glycine/HCl buffer at pH 2.0/0.02% azide (▼▽), human mucus dialyzed for 24 h at 4°C against 0.2 M mercaptoethanol in 0.2 M disodium hydrogen phosphate/sodium dihydrogen phosphate buffer at pH 8.0/0.02% azide (▲△), or human mucus dialyzed for 24 h at 37°C with papain (20 μg/ml) against 0.1 M potassium dihydrogen phosphate/disodium hydrogen phosphate buffer at pH 6.5/0.002 M cysteine/0.005 M ethylenediaminetetraacetic acid (■□).

frequency and the storage modulus (13–21 Pa) was substantially greater than the loss modulus (1.8–3.2 Pa), where both had values comparable to the normal distribution found for pig gastric mucus. Tan δ at 1 rad/s had values in the range 0.14–0.17. The mechanical behavior of mucus taken from the pig duodenum (first 30 cm from the pylorus) was the same as that for human and pig gastric mucus (Figure 3). For all eight samples studied (from eight different duodenums) the moduli showed minimal frequency dependence and the storage modulus (49–72) was substantially greater than the loss modulus (7.5–11.4). The values for the ratio tan δ were between 0.15 and 0.16 throughout the frequency range accessed.

The total concentration of glycoprotein in the two samples of human gastric mucus were 41.0 and 42.3 mg/ml. The total concentration of glycoprotein in pig duodenal mucus varied from 21.4 to 36.2 mg/ml with a mean of 31.8 mg/ml and the protein content ranged from 9.6 to 14.7 mg/ml with a mean of 13.3 mg/ml.

Effects of Proteolysis and Reduction on Native Mucus

After digestion at 37°C of human gastric mucus with papain (24 h) or pig gastric and duodenal mucus with pepsin (10 h), the mucus gel was fully

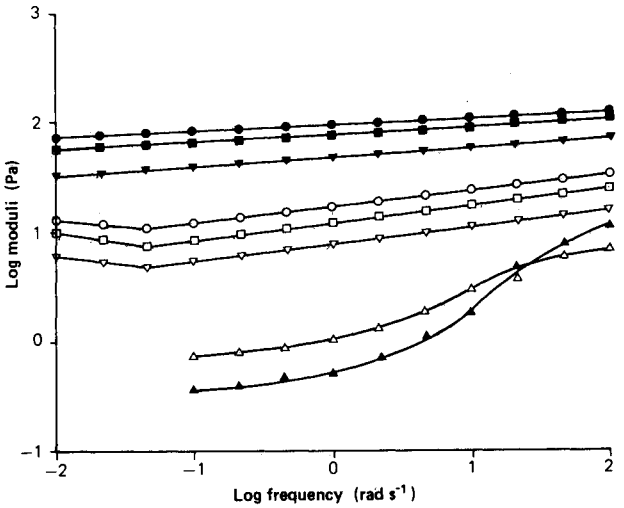

Figure 3. Mechanical properties of pig duodenal mucus: plots of the storage modulus (*closed symbols*) and loss modulus (*open symbols*) against frequency for a typical sample of fresh mucus (●○), mucus dialyzed for 24 h at 4°C against 0.2 M NaCl/0.02% azide adjusted to pH 1.0 with HCl (■□), mucus dialyzed for 24 h at 4°C against 2.0 M NaCl/0.02% azide (▼▽), or mucus gel incubated for 4 h at 37°C with 0.2 M mercaptoethanol in 0.2 M disodium hydrogen phosphate/sodium dihydrogen phosphate buffer at pH 8.0/0.02% azide (▲△).

solubilized; this was accompanied by a large decrease in the measured values of the storage and loss moduli, particularly at lower frequencies. The pattern of frequency dependence of both moduli was also completely changed (Figures 1–3). Both moduli became strongly frequency dependent, and the spectra showed a "cross-over" from a weak, viscoelastic gel (storage > loss) at high frequency to viscous flow (loss > storage) at low frequency, behavior typical of a polymer solution (15,26). A similar complete breakdown in the mechanical properties of the mucus gel was seen after reduction for 24 h by dialysis against 0.2 M mercaptoethanol.

After proteolytic digestion or reduction, tan δ was >1 over the frequency range 10^{-2}–10^{2} rad/s (i.e., predominantly liquidlike response) compared with values <1 for the undegraded mucus gel, thus providing a useful method for following the time-course of the collapse of mucus structure (Figure 4). It was found that the appearance of both pig gastric and duodenal mucus gel did not visibly change when incubated at 37°C in 0.2 M mercaptoethanol for 50–60 min, and the mechanical properties showed little change in relative values of the storage and loss moduli. Between 50 and 90 min a sudden collapse of gel structure occurred, and the sample rapidly assumed the appearance and properties of a viscous liquid. This was shown quantitatively by an increase in tan δ (Figure 4) accompanied by a corresponding rapid increase in frequency dependence of the moduli. A similar time-course study was attempted with pepsin, but proved impractical because the enzyme did not penetrate the gel, but attacked the surface only. The result was that the mucus sample, although decreasing in size (from 1.5 ml), still gave the normal rheologic profile of the native material until the residual mucus gel was too small (after about 2.5–4 h) to give measurable torque between the cone and plate of the instrument.

Proteolytic enzymes, including pepsin, papain, and pronase, have been shown to solubilize pig gastric mucus ($M_r \sim 2 \times 10^6$), with degradation of the glycoprotein into subunits of lower molecular weight ($M_r \sim 5 \times 10^5$) (13). On gel filtration on Sepharose 2B, glycoprotein samples from all untreated mucus samples (human and pig gastric and pig duodenal) eluted as a single excluded peak with a small shoulder extending into the included volume and coeluting with a totally included peak of protein (Figure 5). The proteolytically digested (24 h, 37°C) and reduced (4 h, 37°C) mucus preparations gave a single, included glycoprotein peak eluting in the position of the standard reduced pig gastric mucus glycoprotein subunit (27). This showed that proteolytic digestion and reduction of the polymeric glycoprotein had gone to completion, producing the component subunits. Shorter periods of incubation resulted in glycoprotein being distributed between the excluded and included fractions.

Effect of Ionic Strength on Native Mucus

Samples of mucus from the stomach and duodenum, taken directly from the mucosal surface, were dialyzed for 24 h at 4°C against 0.2 M NaCl, 2 M

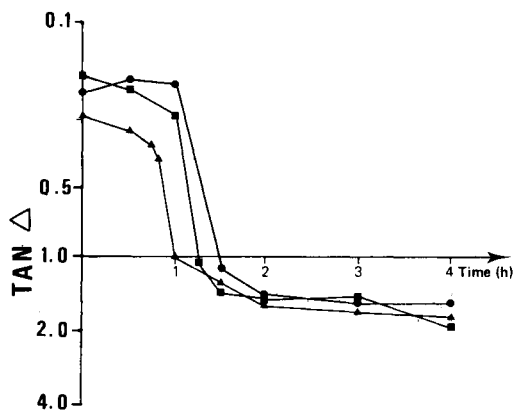

Figure 4. Time-course for the reduction in 0.2 M mercaptoethanol of pig gastric and duodenal mucus and mucus reconstituted from gastric glycoprotein: the ratio of loss to storage modulus, tan δ (1 rad/s and 20% strain), for gastric mucus (▲), duodenal mucus (●), and mucus reconstituted from the purified gastric glycoprotein (■). Samples reduced at 37°C for varying time intervals. The reducing buffer was 0.2 M mercaptoethanol in 0.2 M disodium hydrogen phosphate/sodium hydrogen phosphate buffer at pH 8.0/0.02% azide.

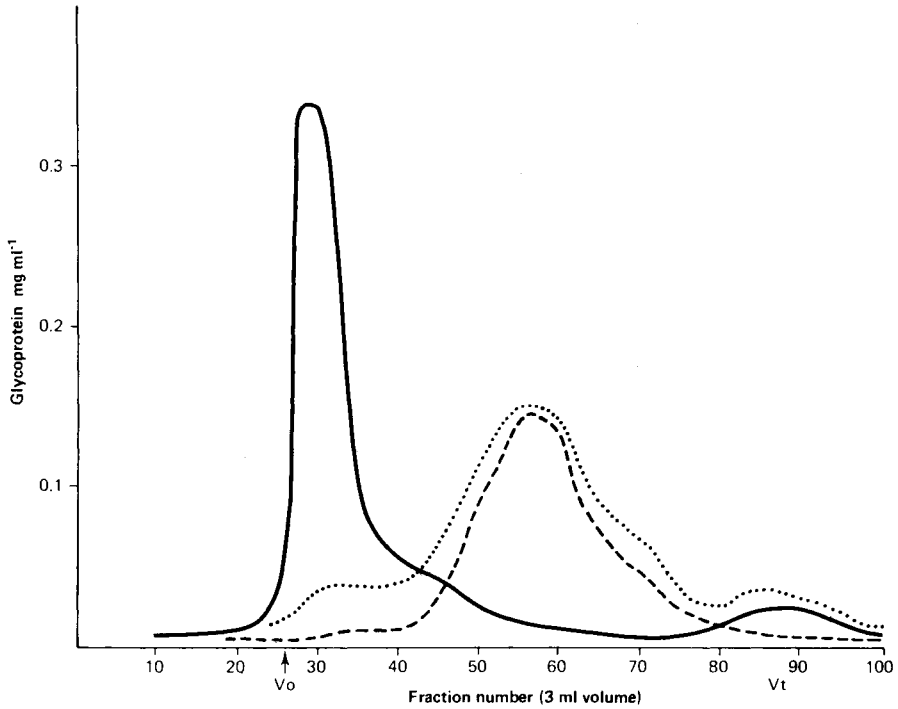

Figure 5. Analysis of pig duodenal mucus by gel filtration on Sepharose 2B of mucus glycoprotein from untreated mucus (*solid line*), mucus glycoprotein from mucus incubated for 10 h at 37°C with pepsin (1 mg/ml) in 0.2 M citric acid adjusted to pH 2.2 with 5 M NaOH (*dashed line*), and mucus glycoprotein from mucus incubated for 4 h at 37°C with 0.2 M mercaptoethanol in 0.2 M disodium hydrogen phosphate/sodium dihydrogen phosphate buffer at pH 8.0/0.02% azide (*dotted line*). Glycoprotein was assayed by the periodic acid-Schiff method (22).

NaCl (containing 0.02% azide), or against distilled water (no azide) (Figures 6 and 7). In each case the gel structure remained intact. The absolute values for the storage and loss moduli remained unchanged with respect to the fresh mucus after treatment with all these agents, nor was there an appreciable change in the ratio tan δ indicating little significant change in gel structure.

Stability of Native Mucus as a Function of pH

The effect of acid on mucus gel structure was investigated by dialyzing fresh samples of native gel for 24 h at 4°C against solutions at pH 1 (0.2 M NaCl/HCl), pH 2 (0.2 M HCl/glycine buffer), pH 5 (0.2 M acetate buffer), and pH 7 or 8 (0.2 M phosphate buffer in both cases). Under these conditions there was no detectable change in mechanical properties even at pH 1, showing that acid per se does not break down mucus gel structure (Figures 2 and 3). These results are expressed in Figures 6, 7, and 8 in terms of tan δ and illustrate that essentially no change had occurred between the control and mucus samples incubated at low pH (4°C). When the experiment was repeated at 37°C (at pH 1–2 for 12 h), however, a significant loss in structural integrity was observed as indicated by a decrease in storage modulus and increased frequency dependence of both moduli. The most likely origin of this behavior is in proteolysis of glycoprotein components by contaminating pepsin (pH optimum ~2.0). Chromatographic analysis on Sepharose 2B of mucus samples that had been incubated under acid conditions at 4°C showed that no detectable breakdown of the glycoprotein (excluded) had occurred, whereas that incubated at 37°C (24 h) had become degraded and was included by the Sepharose gel, evidence that proteolytic cleavage had taken place.

Effect of Bile and Bile Salts on Pig Gastric and Duodenal Mucus

Fresh samples of pig gastric mucus gel were left in direct contact with or dialyzed against one of the following solutions for 18 h at 4°C: undiluted pig gallbladder bile, sodium taurocholate (20 mM), or sodium glycocholate (20 mM). Separate experiments were performed with solutions of each of the above, buffered at pH 8 (0.2 M phosphate), pH 6 (0.2 M phosphate), or pH 2 (HCl/KCl). At pH 2 some precipitation of the bile components occurred over the 18 h. A further series of experiments were performed where both gastric and duodenal gel were incubated for 24 h at 25°C in direct contact with untreated bile from the pig gallbladder. The mucus gel did not dissolve during any of these procedures.

Exposure to gallbladder bile or bile salts produced

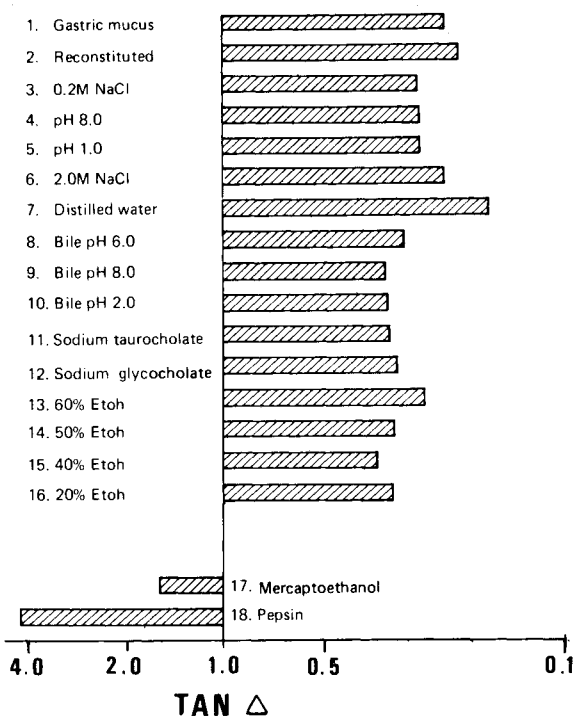

Figure 6. Effects of agents on the mechanical profile of pig gastric mucus: comparison of the ratio of the loss to the storage modulus, tan δ (1 rad/s and strain 20%), for (1) fresh gastric mucus, (2) reconstituted mucus prepared by concentration of the purified mucus glycoprotein (50 mg/ml), (3) mucus dialyzed for 24 h at 4°C against 0.2 M NaCl/0.02% azide, (4) mucus dialyzed for 24 h at 4°C against 0.2 M disodium hydrogen phosphate/sodium dihydrogen phosphate buffer at pH 8.0/0.02% azide, (5) mucus dialyzed for 24 h at 4°C against 0.2 M NaCl adjusted to pH 1.0 with HCl/0.02% azide, (6) mucus dialyzed for 24 h at 4°C against 2.0 M NaCl/0.02% azide, (7) mucus dialyzed for 24 h at 4°C against distilled water, (8) mucus incubated for 24 h at 25°C with pig gallbladder bile at pH 6.1, (9) mucus dialyzed for 24 h at 25°C with pig gallbladder bile at pH 8.0, (10) mucus dialyzed for 18 h at 4°C against pig gallbladder bile adjusted to pH 2.0 with 0.2 M potassium chloride/HCl/0.02% azide, (11) mucus dialyzed for 18 h at 4°C against 20 mM sodium taurocholate in 0.2 M sodium dihydrogen phosphate/disodium hydrogen phosphate buffer at pH 8.0/0.02% azide, (12) mucus dialyzed for 18 h at 4°C against 20 mM sodium glycocholate in 0.2 M sodium dihydrogen phosphate/disodium hydrogen phosphate buffer at pH 8.0/0.02% azide, (13) mucus incubated for 1.5 h at 37°C with 60% ethanol/0.02% azide, (14) mucus incubated for 1.5 h at 37°C with 50% ethanol/0.02 M azide, (15) mucus incubated for 1.5 h at 37°C with 40% ethanol/0.02% azide, (16) mucus incubated for 1.5 h at 37°C with 20% ethanol/0.02% azide, (17) mucus incubated for 4 h at 37°C with 0.2 M mercaptoethanol in 0.2 M disodium hydrogen phosphate/sodium dihydrogen phosphate buffer at pH 8.0/0.02% azide, (18) mucus incubated for 10 h at 37°C with pepsin (1 mg/ml) in 0.2 M citric acid adjusted to pH 2.2 with 5 M NaOH.

no noticeable change in the frequency dependence of the storage and loss moduli (e.g., Figure 1) or tan δ relative to the fresh mucus or the control samples incubated at the same pH in buffer alone (Figures 6 and 7). There was some reduction in the absolute values of both moduli, particularly in gel preparations incubated at pH 8 with sodium glycocholate or sodium taurocholate (up to 40% lower than the control) or bile (16% lower than the control). In all cases, however, the moduli remained within the range found for untreated samples of native gel and values for their ratio, tan δ, were unchanged. Thus mechanical properties and solubility show no evidence of a collapse in mucus gel structure such as that which accompanies the breakdown of mucus structure on proteolysis or reduction. Gel filtration (Sepharose 2B) of the glycoprotein from mucus gels after incubation with bile or its salts showed no

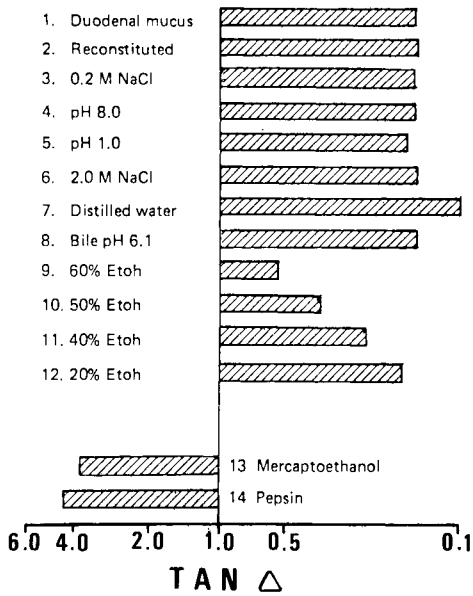

Figure 7. Effects of agents on the mechanical properties of pig duodenal mucus: a comparison of the ratio of the loss to the storage modulus, tan δ (1 rad/s and 20% strain), for (1) fresh duodenal mucus, (2) reconstituted mucus prepared by concentration of the purified mucus glycoprotein (40 mg/ml), (3) mucus dialyzed for 24 h at 4°C against 0.2 M NaCl/0.02% azide, (4) mucus dialyzed for 24 h at 4°C against 0.2 M disodium hydrogen phosphate/sodium dihydrogen phosphate buffer at pH 8.0/0.02% azide, (5) mucus dialyzed for 24 h at 4°C against 0.2 M NaCl adjusted to pH 1.0 with HCl/0.02% azide, (6) mucus dialyzed for 24 h at 4°C against 2.0 M NaCl/0.02% azide, (7) mucus dialyzed for 24 h at 4°C against distilled water, (8) mucus incubated for 24 h at 25°C with pig gallbladder bile at pH 6.1, (9) mucus incubated for 1.5 h at 37°C with 60% ethanol/0.02% azide, (10) mucus incubated for 1.5 h at 37°C with 50% ethanol/0.02% azide, (11) mucus incubated for 1.5 h at 37°C with 40% ethanol/0.02% azide, (12) mucus incubated for 1.5 h at 37°C with 20% ethanol/0.02% azide, (13) mucus incubated for 4 h at 37°C with 0.2 M mercaptoethanol in 0.2 M disodium hydrogen phosphate/sodium dihydrogen phosphate buffer at pH 8.0/0.02% azide, (14) mucus incubated for 10 h at 37°C with pepsin (1 mg/ml) in 0.2 M citric acid adjusted to pH 2.2 with 5 M NaOH.

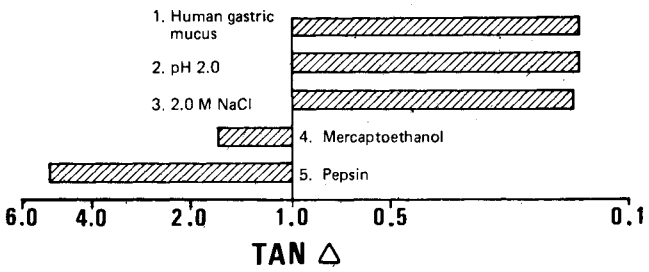

Figure 8. Effects of agents on the mechanical properties of human gastric mucus: comparison of the ratio of the loss to storage modulus, tan δ (1 rad/s and strain 20%), for (1) human gastric mucus, (2) human mucus dialyzed for 24 h at 4°C against 0.2 M glycine/HCl, pH 2.0/0.02% azide, (3) human mucus dialyzed for 24 h at 4°C against 2.0 M NaCl/0.02% azide, (4) human mucus dialyzed for 24 h at 4°C against 0.2 M mercaptoethanol in 0.2 M disodium hydrogen phosphate/sodium dihydrogen phosphate buffer at pH 8.0/0.02% azide, (5) human mucus incubated for 10 h at 37°C with pepsin (1 mg/ml) in 0.2 M citric acid adjusted to pH 2.2 with 5M NaOH.

degradation into fragments of lower molecular weight, and the glycoprotein-positive material was excluded from the column, as that from fresh mucus.

Effect of Ethanol on Pig Gastric and Duodenal Mucus

When gastric and duodenal mucus were incubated for 90 min at 25°C with ethanol up to concentrations of 40% vol/vol there was no detectable change in the mechanical properties (Figures 6 and 7). With 50% and 60% ethanol for 90 min at 25°C varying effects were obtained. For gastric mucus there was a noticeable rise in the values of the storage and loss moduli (130%) but no change in the ratio of the two, tan δ. For duodenal mucus there was also a noticeable rise in both moduli (290%), but this rise was greatest for the loss modulus resulting in a corresponding increase in the ratio tan δ (Figure 7).

Properties of Mucus Gel Reconstituted From Purified Glycoprotein

The pig gastric and duodenal mucus gels were solubilized by homogenization (Waring blender; 1 min) and separated from the remaining nongelatinous cell debris to give the soluble mucus. The soluble mucus was purified by two successive fractionations by equilibrium centrifugation in a CsCl density gradient that gave a glycoprotein preparation containing no detectable contaminant protein or nucleic acid.

Preparations were concentrated by vacuum dialysis to form reconstituted mucus gels at glycoprotein concentrations similar to that in native mucus (about 50 mg/ml and 40 mg/ml for gastric and duodenal mucus, respectively). These reconstituted gels were similar in visual appearance to native mucus, although they were clear, whereas fresh mucus preparations had a faint brown to green coloration. This was possibly due to the absence in the reconstituted mucus of free protein present in the native mucus. All of these reconstituted mucus gels gave mechanical spectra similar to those for the native mucus gel (Figures 1, 6, and 7). The storage modulus was greater than the loss modulus throughout the entire range of frequency (10^{-2}–10^{2} rad/s) and strain (5%–100%) accessed, and there was no sudden drop in dynamic shear moduli with increasing strain such as would accompany rupture of the mucus gel structure. Values of the storage and loss moduli for the reconstituted gels spanned a similar range to those for native mucus. The values of the ratio tan δ for the reconstituted gels were also similar to those for the native gel (Figures 6 and 7).

These results show that mucus gels reconstituted by concentration of the glycoprotein, isolated free of contaminant protein, are closely similar in mechanical properties, and therefore presumably in structure, to native mucus gel taken directly from the surface of the mucosa. The mechanical spectra of the reconstituted mucus gels from both pig gastric and duodenal mucus were unaffected after dialysis against 2 M NaCl or 0.2 M NaCl/HCl at pH 1 for 24 h, but there was a collapse of gel structure after proteolysis with pepsin at 37°C (gastric glycoprotein) and papain at 25°C (duodenal glycoprotein) or reduction with mercaptoethanol at 37°C (Figure 4).

Discussion

The rheologic properties of mucus gels from the human stomach, pig stomach, and duodenum measured by mechanical spectroscopy are characteristic of a weak, viscoelastic gel that is stable and water insoluble. This behavior is defined in terms of the storage modulus being substantially greater than the loss modulus and both having a small but detectable frequency dependence over the frequency range 10^{-2}–10^{2} rad/s (15). This behavior contrasts with that of a superviscous solution resulting from a high concentration of physically entangled polymer (e.g., hyaluronic acid). Although such a preparation visually resembles mucus its mechanical spectrum, over the same frequency range, would show a much greater frequency dependence and at low frequencies the viscous properties (loss > storage moduli) would predominate (26). Alternatively, for a rigid gel (e.g., agar) the pattern of the moduli is not dissimilar to that for mucus, showing essentially no frequency dependence, but the storage modulus is substantial-

ly higher with a corresponding increase in its ratio to the loss modulus.

These rheologic studies are compatible with direct observations of the adherent mucus on ex vivo mucosal sections (8,9). Mucus is observed as a translucent, gelatinous layer forming a continuous cover over the mucosa of variable thickness; values for mean thickness in the rat stomach and duodenum are 73 μm and 82 μm, respectively, and for human gastric antrum the mean mucus thickness is 192 μm. The thickness of the mucus gel layer on the sections does not change over a period of 30 min, evidence that the gel is relatively stable. Mucus, however, will flow and anneal if sectioned, due to the making and breaking in a finite time-scale of noncovalent interactions forming the gel matrix. Mechanical spectra are consistent with such behavior and give an estimate of a time-scale of many minutes, ~10–120 min, for manifestation of flow properties. The observable minimum lifetime for these gel-forming noncovalent interactions is indicated by frequency scans of the moduli (e.g., Figures 1–3) where the elastic response is dominant even at the lowest frequency accessed (10^{-2} rad/s), which corresponds approximately to one oscillatory cycle per 10 min. Restoration of the mechanical properties of mucus gels after mechanical damage, e.g., by freezing and thawing, however, is complete within 2 h, setting an upper limit on the time-scale for structural rearrangement of the mucus gel.

Mucus gel obtained by scraping the surface of the gastroduodenal mucosa contained, besides the glycoprotein, substantial amounts of protein (30%–50% by weight) and a little nucleic acid (<5% by weight). Various workers (28,29) have suggested that some of this protein has a structural role in mucus gel in addition to the glycoprotein. Our studies do not support this. Thus mucus gel reconstituted from the purified glycoprotein, free of protein, had the same mechanical properties as native mucus (for both pig stomach and duodenum). The range of glycoprotein concentration for the reconstituted mucus gels was within that for the fresh mucus and the mechanical properties did not change over these concentration ranges. In dilute solution the viscosity of the glycoprotein from pig gastric and small intestinal mucus is also enhanced by the removal of noncovalently bound protein (30).

The visually more homogeneous appearance of mucus gels reconstituted from purified glycoprotein, in comparison with native mucus, may well reflect the absence of protein contaminants. In the starved animal, adherent mucus is observed as a clear gel, usually without visible cellular contamination (8). Much of the free protein present in native mucus from the mucosal surface may arise from epithelial cells removed during the scraping procedure, although in vivo mucus will inevitably contain secretions such as secretory immunoglobulin A and pepsin (6,31) together with strongly bound lipid components (32).

The mechanical properties for human gastric, pig gastric, and pig duodenal mucus would indicate that the mucus gel structure formed by the component glycoprotein molecules must be essentially the same. In particular, the same mechanical properties observed for human and pig gastric mucus (Figures 1 and 2) confirm the validity of regarding pig mucus as a good model for human mucus, as previously suggested from structural studies (27). Rheologic investigations of pig colonic and small intestinal mucus (33) also indicate much the same structure as in the gastroduodenal mucus gel secretions studied here, although this does not imply that such properties as permeability need also be similar in these gels. Respiratory mucus also has the properties of a weak, viscoelastic gel but with a greater frequency dependence of the moduli than for gastrointestinal mucus (16,17), suggesting that respiratory mucus has more tendency to flow.

The nature of the gel-forming noncovalent interactions between constituent glycoprotein molecules is unknown, but recent evidence for pig gastric mucus (19) suggests that such interactions are between the glycosylated regions of the glycoprotein, possibly by stable interdigitation of the carbohydrate chains. Wide differences in sugar and protein composition as well as in carbohydrate chain length between the mucus glycoproteins from the pig gastrointestinal tract can evidently occur without affecting the ability of these glycoproteins to form mucus gels. In all cases, however, the isolated glycoprotein molecules have a polymeric structure, the integrity of which is necessary for gel formation (6).

A complete change in the mechanical spectra to ones characteristic of a viscous solution was observed after proteolysis or reduction of all three mucus gel secretions (Figures 1–4 and 6–8). This confirms, as previously deduced from viscosity studies in dilute solution (6,13), that the polymeric structure of the glycoprotein is necessary for gel formation. Cleavage of the polymeric glycoprotein into its component subunits as shown by gel filtration (e.g., Figure 5) results in disintegration of the mucus gel structure.

The time-course of collapse of mucus gel structure on incubation in 0.2 M mercaptoethanol shows short-term resistance to mucolysis, with no evidence of any change in mucus gel structure within the first 50–60 min (Figure 4). These results are supported by direct observation of the adherent mucus on mucosal sections in vitro (8) that showed that the thickness of

the mucus gel was unchanged after incubation in *N*-acetylcysteine (20% wt/vol; 30 min; 37°C; pH 2.5 and 7.4).

Addition of *N*-acetylcysteine (5%–20%) has been shown to cause dissipation of the pH gradient at the mucosal surface both in vivo (34) and in vitro (35) within 5–10 min of administration. This effect has been attributed to disruption of the mucus layer supporting the pH gradient. Our results on the rate of solubilization of mucus by thiol agents do not support this explanation for the short-term effect of *N*-acetylcysteine and indicate that it must be acting by some other mechanism. One possible explanation may be found in the adverse effect of *N*-acetylcysteine on the mucosal cells, particularly at hypertonic concentrations (20%, ~1.8 M). A marked reduction (≤50%) has been observed in mucus thickness after addition of 20% *N*-acetylcysteine for 3 min, as measured by the slit lamp and pachymeter (36). Again, our results do not agree with the explanation that this reflects an immediate and direct collapse of the adherent mucus gel. Values for thickness obtained from pH gradients and pachymeter measurements are substantially greater (fourfold to tenfold and twofold to threefold, respectively) than those from direct observation of the adherent mucus gel (8). It has been proposed that these differences are caused by the inclusion of a region of unstirred solution exterior to the adherent mucus gel itself in the pH gradient and pachymeter measurements (37).

It is not possible to assay by mechanical spectroscopy the rate of breakdown of the mucus by pepsin because the enzyme does not penetrate the mucus gel but acts only at the surface of the sample (5). The result is a progressive reduction in size (from 1.5 ml volume) until the amount of mucus gel is so small that it no longer provides a torque between the cone and the plate of the mechanical spectrometer. A rough calculation from the time taken (2.5–4 h) to solubilize 1.5 ml, assuming pepsinolysis of the mucus gel is proportional to its surface area, gives an approximate rate of about 50–100 μm/10 min. Studies on the adherent mucus in vitro show that pepsin (1 mg/ml) removed much of the mucus layer (70–80-μm mean thickness) in 20 min (8).

The effectiveness of the mucus barrier in vivo will depend on two factors: (a) the depth or thickness of the adherent mucus layer and (b) the integrity of its structure. One factor that will affect both of these in vivo is proteolysis. It is clear that pepsin must be a major factor in the breakdown of the mucus gel, for although luminal pepsin cannot diffuse through the mucus adherent to the mucosal surface, it will continually hydrolyze the mucus gel at its luminal aspect to release degraded glycoprotein into the lumen. The thickness of the mucus layer will therefore reflect a dynamic balance between mucus secretion and erosion by pepsin and mechanical forces (5, 6).

The mucus gel can be viewed as consisting of two structural features. First, the covalent polymeric structure of the glycoprotein itself which is destroyed on proteolysis or reduction with loss of gel structure and second, noncovalent interactions between these polymeric glycoprotein molecules that form the mucus gel matrix. These interactions between the glycoprotein molecules that form the mucus gel matrix are resistant to a variety of mucosal damaging agents. Thus prolonged exposure of fresh mucus, or mucus reconstituted from the isolated glycoprotein, to hypertonic NaCl, ethanol (up to 40% vol/vol), bile, and acid (pH 1 to 2) did not alter the mechanical properties of the mucus, and the values of the storage and loss moduli and tan δ were all within the range for fresh mucus (Figures 6–8). After exposure to ethanol concentrations of 50% and 60% (vol/vol) for 90 min, changes were seen in the mechanical properties of the mucus. There was a rise in the values of both moduli and, although the ratio tan δ was unchanged for gastric mucus, there was a distinct rise in that for duodenal mucus. These effects can be explained by dehydration of the mucus in ethanol, which in the case of the duodenum is also associated with some loss of structure. Ethanol concentrations above 80% (vol/vol) cause rapid denaturation of the mucus. A 60%–70% decrease in the thickness of the adherent mucus is observed when rat gastric mucosa is exposed in vitro to 20%–60% (vol/vol) ethanol for 1 h at 37°C (8). Topical administration of absolute ethanol (0.5 ml) to rats in vivo does not, however, cause a significant change in mucus thickness over the mucosa, and breakdown of the mucus barrier is strictly confined to sites of deep-seated mucosal damage identified by overt lesions (9). Other factors in vivo, such as rapid gastric emptying and dilution of the ethanol with the digestive juices, must presumably account for the lack of damage to the existing adherent mucus after the administration of absolute ethanol.

The studies described here show that bile or its salts (sodium taurocholate and sodium glycocholate) do not break down the structure of the mucus gel. When exposed to bile for 18 h, at concentrations in excess of those that are likely to occur in vivo, and over a range of pH 2 to 8, the mechanical properties of gastric mucus are not markedly changed. There is also no change in the mechanical properties of duodenal mucus exposed to bile for 24 h. Although there are small decreases in the values of the moduli, the pattern of their variation with frequency and the

ratio of the storage and loss moduli, tan δ, are unaltered (Figures 1, 6, and 7). In particular, there was no indication of a change that would signify the start of a collapse in gel structure such as that seen on proteolysis or reduction. Bile will cause mucosal damage and biliary reflux has been implicated as a causative factor in peptic ulceration (20,21,38). A proposed explanation for this, based on other rheologic studies, has been the destruction of mucus structure by the bile (39). However, in the latter study respiratory mucus was used, the changes observed were small, and there was no control to show to what extent the changes in rheologic properties were associated with the collapse of mucus gel structure. It is possible that the small changes in mechanical properties observed were due to effects on protein associated with the mucus and present in the unfractionated respiratory material (29). The primary site of action of bile on the mucosa must presumably be at the level of the cells themselves, after first diffusing through the surface mucus layer. The permeability of small intestinal mucus has been shown to be unchanged by bile salts (40).

Our present studies on isolated mucus gel, together with previous observations of adherent mucus on ex vivo mucosal sections (9,10), show that in the short term the structure of the mucus barrier is resistant to abuse by hypertonic NaCl, bile, pH 1 to 2, ethanol, and indomethacin. However, the thin layer of adherent mucus is readily permeable to ions and small molecules and will not prevent the rapid penetration of these agents to the underlying epithelial cells. Under such conditions extensive epithelial cell damage is shown to occur followed by rapid restitution of the surface epithelium (41–43). The mucus barrier remains for at least 1 h after exposure to the damaging agents in vivo (9, 10). This, together with the disrupted epithelial cells, would form a barrier that would still restrict the mixing of the buffered contents of cells and interstitial fluid with bulk acid in the lumen, maintain a barrier to pepsin, delay further disruption of the epithelium, and thus facilitate epithelial restitution. If the mucus layer were not there it could be anticipated that the damage from these agents would be more severe. However, erosion of the mucus layer by pepsin and mechanical sloughing will continue and, unless this is balanced by continued mucus secretion from viable cells, breakdown of the mucus barrier will occur.

References

1. Hollander F. The two-component mucus barrier. Arch Intern Med 1954;93:107–20.
2. Heatley NG. Mucosubstance as a barrier to diffusion. Gastroenterology 1959;37:313–8.
3. Allen A, Garner A. Gastric mucus and bicarbonate secretion and their possible role in mucosal protection. Gut 1980; 21:249–62.
4. Flemström G, Garner A. Gastroduodenal HCO_3^- transport: characteristics and proposed role in acidity regulation and mucosal protection. Am J Physiol 1982;242:G183–93.
5. Allen A. The structure and function of gastrointestinal mucus. In: Harmon JW, ed. Basic mechanisms of gastrointestinal mucus. Baltimore, London: Williams & Wilkins:1981;351–7.
6. Allen A. The structure and function of gastrointestinal mucus. In: Johnson LR, ed. Physiology of the gastrointestinal tract. Vol. 1. New York: Raven, 1981;617–39.
7. Florey H. Mucin and the protection of the body. Proc R Soc Lond B 1955;143:144–8.
8. Kerss S, Allen A, Garner A. A simple method for measuring thickness of the mucus gel layer adherent to rat, frog and human gastric mucosa: influence of feeding, prostaglandin, N-acetylcysteine and other agents. Clin Sci 1982;63:187–95.
9. McQueen S, Hutton D, Allen A, Garner A. Gastric and duodenal surface mucus gel thickness in rat: effects of prostaglandins and damaging agents. Am J Physiol 1983;245:G388–93.
10. McQueen S, Allen A, Garner A. Measurement of gastric and duodenal mucus gel thickness. In: Allen A, Flemström G, Garner A, Silen W, Turnberg L, eds. Mechanism of mucosal protection in the upper gastrointestinal tract. New York: Raven, 1983;215–9.
11. Bell AE, Allen A, Morris E, Rees D. Rheological studies on native pig gastric mucus gel. In: Chantler EN, Elder JB, Elstein M, eds. Adv Exp Med Biol 1982;144:97–9.
12. Allen A, Snary D, Pain RH. Model for the structure of the gastric mucus gel. Nature 1976;264:88–9.
13. Snary D, Allen A, Pain RH. Structural studies on gastric mucoproteins: lowering of molecular weight after reduction with 2-mercaptoethanol. Biochem Biophys Res Commun 1970; 40:844–51.
14. Scawen M, Allen A. The action of proteolytic enzymes on the glycoprotein from pig gastric mucus. Biochem J 1977;163: 363–8.
15. Ferry JD. Viscoelastic properties of polymers. New York: John Wiley & Sons, 1980.
16. Khan MA, Wolf DP, Litt M. Effect of mucolytic agents on the rheological properties of tracheal mucus. Biochem Biophys Acta 1976;444:369–73.
17. King M. Viscoelastic properties of airway mucus. Fed Proc 1980;39(12):3061–80.
18. Meyer F, Silberberg A. The rheology and molecular organisation of epithelial mucus. Biorheology 1980;17:163–8.
19. Bell AE, Allen A, Morris E, Ross-Murphy S. Gel-forming interactions in pig gastric mucous glycoprotein. Trans Biochem Soc 1984;12:648.
20. Davenport HW. Physiological structure of the gastric mucosa. In: Code CF, ed. Handbook of physiology: alimentary canal. Vol. 2. Baltimore: Williams & Wilkins, 1967;759–79.
21. Fromm D. Gastric mucosal barrier. In: Johnson RL, et al., eds. Physiology of the digestive tract. New York: Raven, 1981; 733–48.
22. Mantle M, Allen A. A colorimetric assay for glycoproteins based on the periodic acid/Schiff stain. Biochem Soc Trans 1978;6:607–9.
23. Creeth JM, Denborough MA. The use of equilibrium density gradient methods for preparation and characterisation of blood group specific glycoproteins. Biochem J 1970;117:879–91.

24. Starkey BJ, Snary D, Allen A. Characterisation of gastric mucoproteins isolated by equilibrium density gradient centrifugation in caesium chloride. Biochem J 1974;141:633–9.
25. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.
26. Morris ER, Ross-Murphy SB. Chain flexibility of polysaccharide and glycoproteins from viscosity measurements. In: Northcote DH, ed. Techniques in carbohydrate metabolism. Vol. B3. Amsterdam: Elsevier, 1981:1–46.
27. Pearson JP, Allen A, Venables CW. Proteolytic digestion of human and pig gastric mucus glycoproteins. In: Schauer R, ed. Glyconjugates. Stuttgart: Georg Thieme, 1979:136.
28. List SJ, Findlay BP, Forstner GG, Forstner JF. Enhancement of viscosity of mucin by serum albumin. Biochem J 1978;175: 565–71.
29. Creeth JM. Constituents of mucus and their separation. Br Med Bull 1978;34:17–24.
30. Mantle M, Allen A. Isolation and characterisation of the native glycoprotein from pig small-intestinal mucus. Biochem J 1981;195:267–75.
31. Forstner J. Intestinal mucins in health and disease. Digestion 1978;17:234–63.
32. Witas H, Slomiany BL, Zdebska E, Kojuma K, Lian YH, Slomiany A. Lipids associated with dog gastric mucus glycoprotein. J Appl Biochem 1983;5:16–24.
33. Sellers LA, Allen A, Morris E, Ross-Murphy SB. Rheological studies on pig gastrointestinal mucous secretions. Trans Biochem Soc 1983;11:763–4.
34. Ross IN, Bahari HMM, Turnberg LA. The pH gradients across mucus adherent to rat fundic mucosa in vivo and the effect of potential damaging agents. Gastroenterology 1981;81:713–8.
35. Takeuchi KD, Magee D, Critchlow J, Silen W. Role of pH gradient of mucus in protection of gastric mucosa. Gastroenterology 1983;84:331–40.
36. Bickel M, Kauffman, GL. Gastric mucus gel thickness: effect of distension, 16,16-dimethyl prostaglandin E_2 and carbenoxolone. Gastroenterology 1981;80:770–5.
37. Allen A, Hutton D, McQueen S, Garner A. Dimensions of gastroduodenal surface pH gradients exceed those of adherent mucus gel layers. Gastroenterology 1983;85:463–76.
38. Rhodes J, Barnardo DE, Phillips SF, Rouelstad RA, Hoffman AF. Increased reflux of bile into the stomach in patients with gastric ulcer. Gastroenterology 1969;57:241–52.
39. Martin GP, Marriott C, Kellaway IW. Direct effect of bile salts and phospholipids on the physical properties of mucus. Gut 1978;19:103–7.
40. Lukie BE. Studies of mucus permeability. Mod Probl Paediat 1977;19:46–53.
41. Svanes K, Ito S, Takeuchi K, Silen W. Restitution of the surface epithelium of the in vitro frog gastric mucosa after damage with hyperosmolar sodium chloride morphologic and physiologic characteristics. Gastroenterology 1982; 82:1409–26.
42. Lacy ER, Ito S. Ethanol induced insult to the superficial rat gastric epithelium: a study of damage and rapid repair. In: Allen A, Flemström G, Garner A, Silen W, Turnberg L, eds. Mechanisms of mucosal protection in the upper gastrointestinal tract. New York: Raven, 1983;49–54.
43. Morris GP, Harding PL. Mechanisms of mucosal recovery from acute damage: roles of extracellular mucus and cell migration. In: Allen A, Flemström G, Garner A, Silen W, Turnberg L, eds. Mechanisms of mucosal protection in the upper gastrointestinal tract. New York: Raven, 1983;209–13.