

Direct effect of bile salts and phospholipids on the physical properties of mucus

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SUMMARY Reflux of duodenal contents into the stomach has been implicated in the disruption of mucosal defence and the subsequent occurrence of gastric ulcer. The change produced in the rheological properties following the addition of bile salts and phospholipids to mucus samples was used to assess resultant structural changes. Sodium deoxycholate, sodium taurodeoxycholate, sodium glycocholate, and lysophosphatidylcholine decreased both viscosity and elasticity, indicating that structural breakdown had occurred, whereas phosphatidylcholine could not be shown to have any effect. It is therefore suggested that some of the ulcerogenic activity of naturally occurring surfactants may be associated with their ability directly to reduce mucus consistency.

It is unlikely that any one sequence of events can adequately explain the initiation of gastric ulcers. Evidence has been provided to support two main hypotheses for the aetiology of gastric ulcer (Rhodes, 1972): one suggests increased acid secretion coupled with a delayed gastric emptying time, while the other suggests that a regurgitation of duodenal contents takes place. In support of the latter hypothesis, several workers have found that the concentrations of bile salt in certain ulcer patients are considerably higher than in normal controls (Du Plessis, 1965; Capper, 1967; Rhodes *et al.*, 1969). A similar rise in the levels of another surfactant present in duodenal juice, lysophosphatidylcholine, has been reported (Johnson and McDermott, 1974). Slomiany *et al.* (1975) have also been able to demonstrate a higher lysophospholipid content in the gastric mucosa of ulcerated rats. In addition, evidence of gastric mucosal breakdown has been provided by measurement of transmucosal ionic fluxes in the presence of bile and lysophosphatidylcholine (Davenport, 1968, 1970).

Du Plessis (1965) suggested that reflux of alkaline duodenal contents may act only by interfering with the protective layer of mucus and thus allow acid and pepsin to come into contact with the epithelium. The purpose of the present study is to determine whether bile salts and phospholipids can modify the physical structure of mucus, as reflected by the change in rheological properties.

Methods

MATERIALS

The bile salts sodium deoxycholate (Koch Light), sodium taurodeoxycholate (Sigma) and sodium glycocholate (Sigma), and the sodium dodecyl sulphate, specially purified for biochemical work (BDH), were used as supplied.

Egg phosphatidylcholine (BDH) was further purified by elution from an alumina column (Kellaway and Saunders, 1969), followed by recrystallization from warm methyl ethyl ketone until a single spot was produced by thin-layer chromatography. The pure compound was stored under dry acetone at 5°C. When required, an acetone dispersion was dried and the phosphatidylcholine dissolved in the minimum amount of ether. Water was added to produce a fine dispersion and the ether evaporated, the last traces of ether being removed by bubbling through nitrogen. A sol, for application to the mucus sample, was prepared by sonication at 20 KHz for 90 minutes (Dawe Soniprobe).

Lysophosphatidylcholine was prepared from egg phosphatidylcholine using a modification of the method of Hanahan *et al.* (1954). The product was recrystallised from hot ethanol and stored under dry acetone at 5°C.

Bronchial mucus collected from hospitalised patients was used as the mucus model. Gastric mucus from pigs was not used because of the small amount of uncontaminated material which could be obtained. Human gastric mucus, although more

homogeneous, was difficult to obtain in sufficient quantities. The biochemical differences between bronchial and gastric mucus are slight (Waldron-Edward and Skoryna, 1970; Yosizawa, 1972; Boat *et al.*, 1976) and do not significantly influence the physical structure of the mucus gel—for example, it has been reported that total removal of the sialic acid does not alter the rheological properties (Meyer *et al.*, 1975). It has also been shown that bronchial and gastric mucus are rheologically similar and that the same drug-induced changes are produced with both systems (Marriott and Kellaway, 1976; Woods, 1977). It was therefore considered justifiable to use bronchial mucus as a model for gastric mucus, since this is available in suitable quantities. The mucus was homogenised by the method reported by Marriott and Richards (1974), so as to minimise rheological variations within a batch. Any samples which were purulent or contained blood were rejected.

Glass distilled water was used throughout.

EXPERIMENTAL TECHNIQUES

Weighed aliquots of homogenised mucus were treated with 10% of their weight of a concentrated solution of the test compound so that the required final concentration resulted in the mucus sample. Control aliquots were treated in a similar manner using distilled water in place of the surfactant solution. After treatment the mucus samples were allowed to equilibrate at 37°C for 30 minutes in order to allow any interaction to take place. At the end of the interaction period, the mucus sample was

transferred to the gap between cone and plate of an air turbine viscometer described by Marriott *et al.* (1973). The sample was allowed to equilibrate for a further 30 minutes in a saturated atmosphere, a period sufficient to relax any stresses applied during the loading procedure. A constant shear stress was then applied and the resultant shear strain recorded with time, to produce a creep curve (Fig. 1).

EXPLANATION OF TERMS

The two extremes of rheological behaviour are the Newtonian fluids, which can be described purely in terms of viscosity—for example, water, silicone oil—and the Hookian solids, which possess solely an elastic component, having no tendency to flow—for example, rubber, steel. Many materials, of which mucus is one, fall between these two extremes, possessing properties of both a Newtonian fluid and a Hookian solid; such materials are termed viscoelastic (Denton *et al.*, 1968). The elastic component cannot be ignored and mucus should not therefore be characterised in terms of viscosity alone. Creep compliance is a suitable method for evaluating these viscoelastic properties (Davis, 1973). In this technique, when a constant shear stress is applied, viscoelastic materials exhibit the type of response shown in Fig. 1.

Initially the mucus stretches elastically, as represented by the instantaneous 'jump' AB. If elasticity is decreased, by the addition of a drug, for example, then AB is increased, since it is usual to describe this

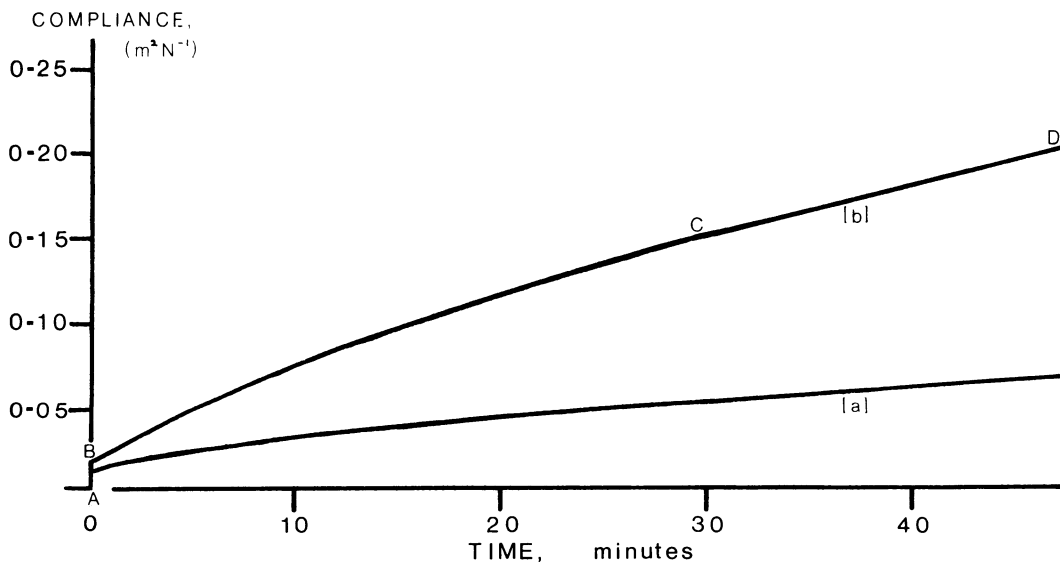


Fig. 1 Typical creep curve of mucus treated with (a) distilled water, (b) 6 mM sodium deoxycholate.

Direct effect of bile salts and phospholipids on the physical properties of mucus

region in terms of an instantaneous shear compliance, J_0 , the reciprocal of elasticity.

In the region BC, which is curved throughout its length, the material exhibits both viscous and elastic behaviour simultaneously; the material is prevented from flowing in a purely viscous manner by the superimposed elastic properties. This behaviour can be characterised in terms of a viscosity coefficient and another elastic modulus.

At longer times the relative contribution of the elastic modulus is reduced and eventually purely viscous behaviour is observed. This is represented by the linear region CD, the slope of which yields the viscosity coefficient η_0 . The mucus is in the rheological ground state, no structural breakdown occurring, hence the viscosities calculated in this region are very high compared with standard methods and they reflect the viscosity of the undestroyed gel.

Since treatment of the mucus with the test com-

pounds used in this study resulted in a decreased gel structure—that is, lower consistency—then the experimental curve for the treated mucus sample (curve b) lies above that of the control (curve a). If structure had been induced—that is, an increase in consistency—then curve b would have been displaced in the opposite direction.

Results

Figure 2 shows the effect of three individual bile salts, sodium deoxycholate, sodium glycocholate, and sodium taurodeoxycholate on the residual shear viscosity, η_0 . As the concentration of bile salt was increased so the viscosity decreased. At the highest concentrations employed (20 mM), liquefaction of the gelled mucus had occurred. The decrease in η_0 produced by bile salts, which can be considered as physiologically occurring anionic surfactants, was directly comparable to that produced by a synthetic anionic, sodium dodecyl sulphate (SDS). Lyso-phosphatidylcholine (LPC) was also found to be mucolytic, as reflected by the decrease in viscosity produced by its addition (Fig. 3). In contrast phos-

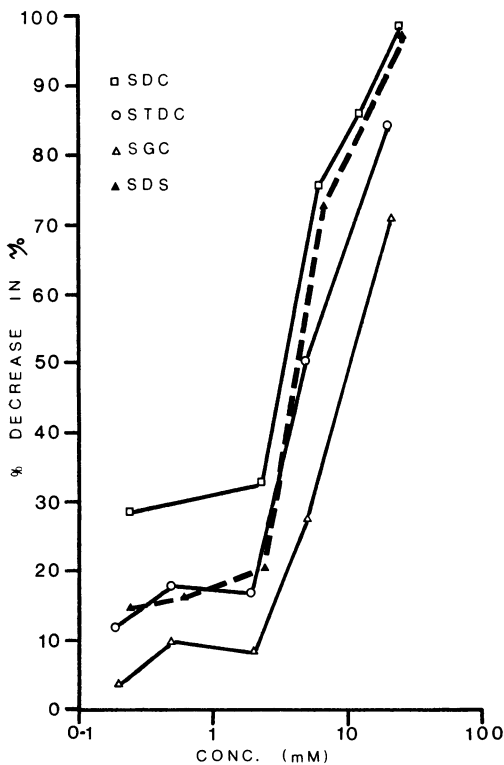


Fig. 2 The change in mucus viscosity on addition of sodium deoxycholate (SDC), sodium taurodeoxycholate (STDC), sodium glycocholate (SGC), and sodium dodecyl sulphate (SDS). All points on this and subsequent figures are the mean of at least three determinations.

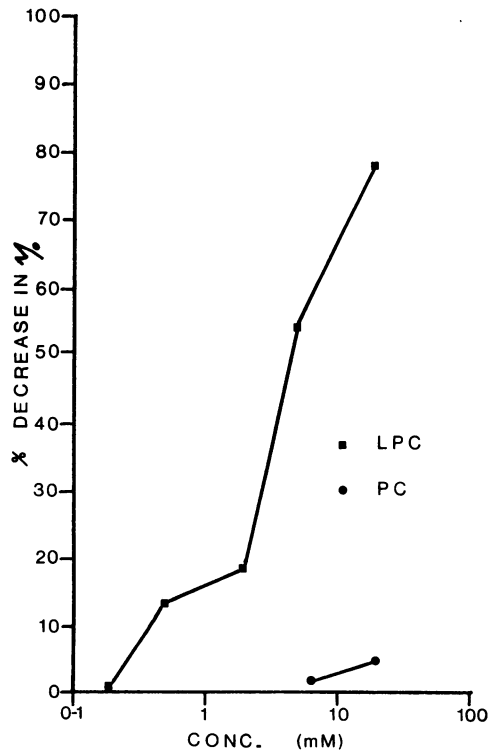


Fig. 3 The change in mucus viscosity on addition of lysophosphatidylcholine (LPC) and phosphatidylcholine (PC).

phatidylcholine (PC), when present at 6 mM and 20 mM, the two highest molar concentrations employed for the bile salts and LPC, did not exert any profound effect on viscosity (Fig. 3).

The effect of bile salts and SDS, for comparison, on instantaneous compliance (the reciprocal of elasticity), J_0 , is shown in Fig. 4. As the concentration of applied bile salt was increased then so the compliance increased—that is, the elasticity decreased. The decrease in elasticity of mucus treated with bile salts at the lower concentration (0.2, 0.5, 2 mM) used in this study was insignificant. SDS, on the other hand, produced measurable changes at such concentrations. The decrease in elasticity at a final bile salt concentration of 20 mM is dramatic (85–370%), although this is to be expected, since, as stated previously, the mucus alters physically in character from a semi-solid to a liquid. LPC produces a similar increase in compliance to the bile salts (Fig. 5), whereas treatment with a 20 mM PC sol had little effect on J_0 .

Since all the surfactants examined, with the exception of PC, decreased both the viscous and elastic parameters, it is clear that the degree of structuring in a mucus gel is decreased by the presence of such compounds.

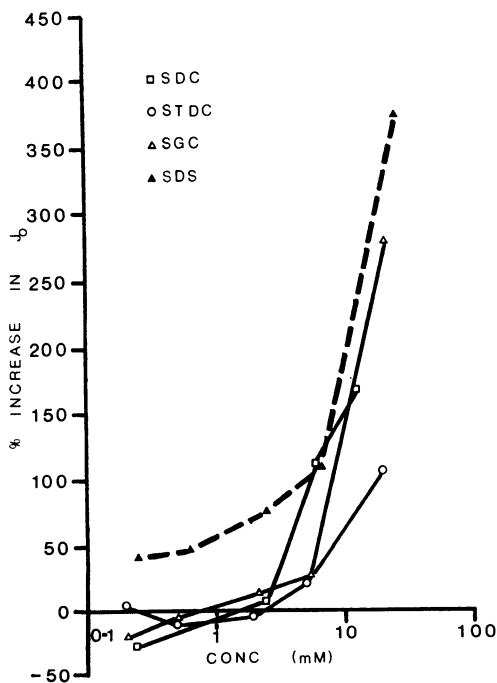


Fig. 4 The change in mucus compliance on addition of either bile salts or anionic surfactant (for abbreviations, see legend to Fig. 2).

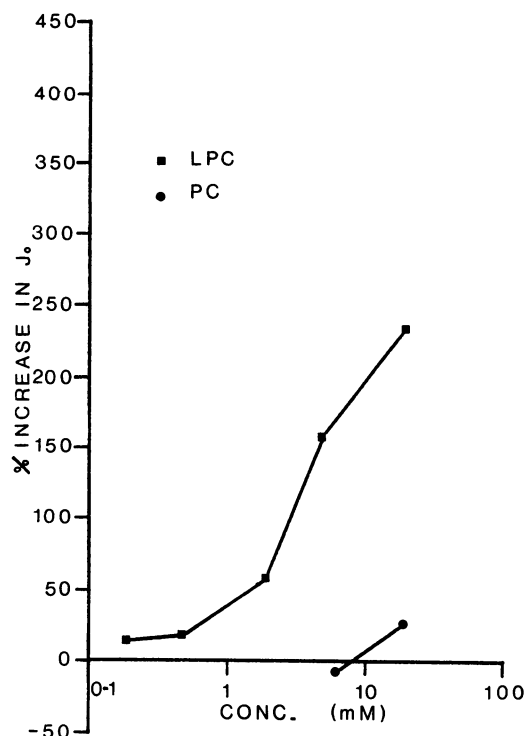


Fig. 5 The change in mucus compliance on addition of phospholipids (for abbreviations, see legend to Fig. 3).

Discussion

If duodenal reflux occurs, the first component of the gastric mucosa that the duodenal contents will encounter is the mucus layer. One of the primary functions of gastric mucus is to prevent the underlying mucosa being exposed to luminal contents. Should structural breakdown of the mucus occur, it may be unable effectively to fulfil this function. It is apparent from this study that bile salts and LPC, surfactants that are present in duodenal juice, can modify the rheological properties of mucus, decreasing both viscosity and elasticity over the concentration range investigated. Black *et al.* (1971) have reported that when the concentration of bile on the mucosa increases then a greater transmucosal hydrogen ionic flux occurs. Such a change could well be associated with the concentration dependent breakdown in mucus structure observed in this work. A 20 mM concentration of both bile salts and LPC produced a decrease in the residual viscosity of between 70 and 100%. Although a concentration of 20 mM is much higher than that found in the lumen by Rhodes and others (1969) for bile salts and Johnson and McDermott (1974) for LPC, it is

possible that such concentrations could be obtained and exceeded locally when reflux occurs.

An almost identical reduction in mucus structure was produced with SDS. This would suggest that the observed mucolytic activity is not confined to the bile constituents but is possibly a general property of anionic surfactants.

PC, in contrast to the other compounds investigated, could not be demonstrated to disrupt gel structure. Most of the PC present in bile, however, is hydrolysed by phospholipase A of pancreatic juice to LPC. The reaction is activated by bile acids and also by trypsin. The lack of effect with PC is therefore insignificant in that most of the refluxed phospholipid will be in the hydrolysed (lyso-) form.

Davenport (1968, 1970) has provided evidence that the gastric mucosal barrier as a whole is destroyed by the presence of bile and lysophosphatidylcholine, resulting in hydrogen ions diffusing from the lumen to the mucosa. Winborn *et al.* (1976) have shown that the presence of bile in the rat stomach causes severe damage to the epithelia, with the occurrence of haemorrhagic areas throughout the mucosa. However, in order to produce such effects the bile contents must first penetrate the protective mucus layer and this would be greatly facilitated by the *in vitro* decrease in gel structure demonstrated in this work. Some evidence that this effect does in fact occur *in vivo* has been provided by Winborn *et al.* (1976) who commented upon the altered appearance of the mucus layer during treatment with bile salts. The consequences of such a modification of the mucus layer would appear to be twofold: (1) the facilitated transport of highly toxic duodenal contents to the mucosa and the subsequent damage to mucosal cells; and (2) the exposure of the gastric mucosa to the acidity of the lumen. Either, or probably both, of these effects could produce gastritis and ultimately gastric ulceration.

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