A mucin model for the *in vitro* evaluation of mucolytic agents

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The sputum produced in asthma and chronic bronchitis is a viscoelastic gel. Potential mucolytic agents are usually assessed by their action on sputum samples collected from patients. Such samples are variable in their properties and meaningful data are difficult to derive. Consequently, we have developed an *in vitro* mucin model based on hog gastric mucin (Sigma). It comprises 10% mucin, which is allowed to hydrate for 12 h at 4°, and the pH is adjusted to 8·0 using tris buffer. Biorheological studies have been conducted using the Ferranti-Shirley cone and plate viscometer and a new stress relaxation method based on a micro-force balance (Davis, 1973). The relative mucolytic activities of a range of commercially available drugs and potential compounds are shown in Table 1.

The reduction in consistency, as compared to a control with water, varied with the agent and its concentration. The relative mucolytic activities found with the model were in agreement with reported *in vitro* and *in vivo* studies using sputum samples (Sheffner & Lish, 1970). The potential mucolytic agent dithiothreitol was ten times more effective than *N*-acetyl cysteine at the same molar concentration. It has been suggested that these two compounds break disulphide bonds in mucoprotein by means of a disulphide-sulphydryl interchange reaction. However, thiourea (also containing a sulphydryl group) is ineffective as a mucolytic agent. The consistency of the mucus model can be increased by small quantities of added disodium tetraborate. This is due to cross-linking of mucoprotein chains.

Table 1. In vitro *reduction in consistency of mucin model compared to control with water (37°).*

Compound	Concn %	% fall in consistency (60 min)	Compound	Concn %	% fall in consistency (60 min)
N-Acetyl cysteine	0·5	39	Superinone	0·0125	—2
	1·0	55	Urea	2·4	—2
	2·0	63	Thiourea	1·0	—1
Dithiothreitol	0·2	62	Disodium Tetraborate	0·1	—45
	2·0	73			
Ascoxal	3·0	18			
Trypsin	0·1	31			

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Effect of mucin on the bioavailability of tetracycline from the gastro-intestinal tract: *in vivo*, *in vitro* correlations

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Functions of the mucus film lining the G.I. tract are poorly understood. This work employed four techniques to investigate the influence of a mucus model (porcine crude mucin) upon the bioavailability of tetracycline from the small intestine. (1) *In vivo* rat intestine perfusion/absorption technique—perfused with tetracycline phosphate (a) in phosphate buffer pH 6·3 and (b) in 1% mucin in buffer, samples taken at 20, 40 and 60 min and analysed for tetracycline. (2) Everted gut method—solutions (a) and (b) analysed as in (1). (3)

Diffusion cell with cellulose acetate membrane—donor solutions (a) and (b), receptor fluid phosphate buffer pH 6·3. After reaching steady state, samples were analysed hourly for 6 h to calculate apparent permeability coefficients. (4) Sartorius Absorption Simulator with artificial intestinal wall—donor solutions (a) and (b), receptor (representing plasma) buffer pH 7·5. Samples analysed hourly for 6 h to calculate Sartorius diffusion rate constant.

Table 1.

Method	*In vivo* perfusion			Everted gut			Diffusion cell	Absorption simulator
Parameter	% App. Absorption			Serosal conc (μg)			Pa ($cm^2\ s^{-1}$)*	kd ($cm\ min^{-1}$)
No. of Replicates	20			12			15	5
Time (min)	20	40	60	20	40	60		
Tetracycline (TC)	17·2 ±1·2**	30·3 ±1·3	43·3 ±1·4	18·1 ±1·7	77·2 ±7·0	202 ±10	$3{\cdot}2 \times 10^{-9}$	$4{\cdot}6 \times 10^{-4}$
TC + Mucin	8·2 ±0·7	16·1 ±0·9	24·1 ±1·2	20·2 ±1·6	45·1 ±2·1	88·8 ±7·2	$2{\cdot}4 \times 10^{-9}$	$2{\cdot}4 \times 10^{-4}$
% Reduction	52	47	45	—10	42	56	23	49

* Pa = Apparent Permeability Coefficient; kd = Sartorius Diffusion Rate Constant.
** Value quoted with error of mean.

The everted gut method was easy, reliable and cheap, but absorption rates were unnaturally slow due to lack of blood supply and other factors, complications not present in the *in situ* rat intestine perfusion method. When using artificial membranes drug transfer rates depend mainly upon the drug partition coefficient between solvent and membrane. The physiological significance of the results can be increased by using the most suitable membrane and conditions e.g. in the Sartorius Absorption Simulator.

If the gel nature of natural mucin arises from overlapping rigid rods of protein-carbohydrate then at low concentrations (little interlocking), the mobile suspension provides little viscous resistance to drug passage. Thus the reduced bioavailability of tetracycline (Table 1) may be due to hydrogen bonding to the mucin e.g. at the many possible sites on the carbohydrate side chains, or to solubilization of the drug in the lipophilic portions of the macromolecule.

The effect of food on the *in vivo* release of propranolol from a PVC matrix tablet in the dog

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Plastic matrix tablets have been used extensively as sustained-release formulations for drugs (Graffner & Sjögren, 1971; D'Arcy, Griffin & others, 1971). Various factors affecting release of drugs from these tablets have been reported (Sjögren, 1971). This communication reports studies carried out to examine the effect of food on the *in vivo* release of propranolol from a sustained-release matrix tablet.

The matrix tablet consisted of propranolol hydrochloride, (Inderal; 125 mg), embedded in an insoluble matrix of Pevikon D–42–P (polyvinyl chloride, 273 mg). This tablet was of sufficient hardness to survive passage through the alimentary tract of a dog intact and released approximately 50% of the dose in 3 h, as measured using the USP dissolution apparatus at 100 rev min^{-1}. Measurement of β-blockade in dogs (using isoprenaline challenge), demonstrated a considerable prolongation of activity compared with a standard propranolol tablet. A single 125 mg matrix tablet maintained greater than 50% blockade over a period of about 24 h.