The influence of mucin on the bioavailability of tetracycline

Saggers & Lawson (1966) reported that tetracycline was strongly bound to hog gastric mucin to form an orange-yellow pigmented solid, and that pre-chelation with ethylenediaminetetraacetic acid reduced the degree of binding to mucin. Albert (1952, 1956) showed that a complex was formed by tetracycline in the presence of metal ions.

The role of a cross linking agent in mucus structure has been attributed to calcium ions (Deman, Mareel & Bruyneel, 1973) although these ions have been shown to have an insignificant effect on the tetracycline-mucus intereaction (Marriott & Kellaway, 1975). An increase in rigidity and viscosity was demonstrated on the addition of tetracycline to homogenized samples of bronchial mucus (Kellaway & Marriott, 1973) which would suggest changes in the structure of the glycoprotein gel networks. Lawson (1967) reported that tetracycline was capable of permeating mucus, although the quantity of drug diffusing did not decrease linearly with mucin concentration. It is considered feasible that the glycoprotein-tetracycline interaction and the changes induced in the gel network will impair absorption of the antibiotic from the gastrointestinal tract.

In this work the aspect of bioavailability examined concerns the possible influence of mucus on the release rate of tetracycline from unit solid dosage forms.

The effect of viscosity on the dissolution rate has recently been examined (Braun & Parrott, 1972). Florence, Elworthy & Rahman (1973) examined the influence of solution viscosity of some hydrophilic polymers on the release rate of soluble salts from compressed discs and concluded that dissolution rate constants could not be related by one equation to the bulk viscosity. An empirical correlation was however achieved when the bulk viscosity was replaced by an effective viscosity.

Discs were prepared by direct compression of a finely powdered mixture of 250 mg potassium chloride (Analar) and 50 mg tetracycline hydrochloride (The Boots Co. Ltd.) at a pressure of 76·5 kg mm^{-2}. The complete dye and disc were fitted into a Perspex former so that only one face of the disc was exposed and the former was then placed on the bottom of a 600 ml beaker. Dispersions of mucin (Sigma Hog Gastric) were prepared by stirring the required weight of the dry powder in water for 10 min and ageing the resulting crude dispersion for 24 h at 5°. 500 ml of water or mucin dispersion at 37° was then added to the beaker and stirred by a twin bladed stainless steel stirrer, situated 35 mm above the centre of the disc, rotating at 112 rev min^{-1}. 0·5 ml samples were withdrawn every 30 s from a fixed sampling point and assayed for tetracycline content at 354 nm. All experiments were repeated twice.

Rate constants were calculated from the Noyes-Whitney equation: $dc/dt = k(c_s - c)$ where c = concentration of dissolving solute at time t; c_s = saturation solubility of solute; k = rate constant, which on expansion and neglecting terms in k greater than the square gives $c/t = kc_s - k^2c_st/2$.

Since the value of the saturation solubility of tetracycline hydrochloride at 37° had been previously determined then the rate constant k could be derived from the intercept of the plot of c/t against t. The viscosity of the dissolution media was determined using a Contraves Rheomat 30 with a Rheoscan programming unit. A sweep time of 120 s and a maximum shear rate of 195 s^{-1} were used.

The rate constant (k) decreased as the gastric mucin concentration was increased to 2% and was inversely proportional to the viscosity for concentrations of mucin below 1·5% (Fig. 1). This relation would be predicted for diffusion-controlled drug release systems. The reason for the deviation of the 2% sample is unknown, although with the stirring conditions used sedimentation of particles from the crude mucin may occur

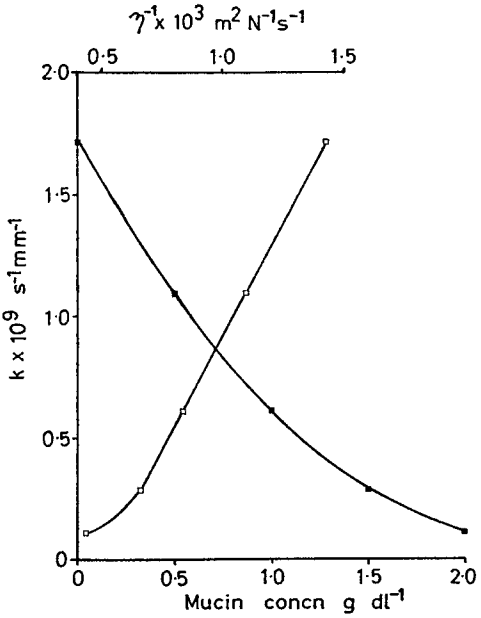

FIG. 1. The rate constant, plotted as a function of mucin concentration (■) and fluidity, η^{-1}, (□) at 37°.

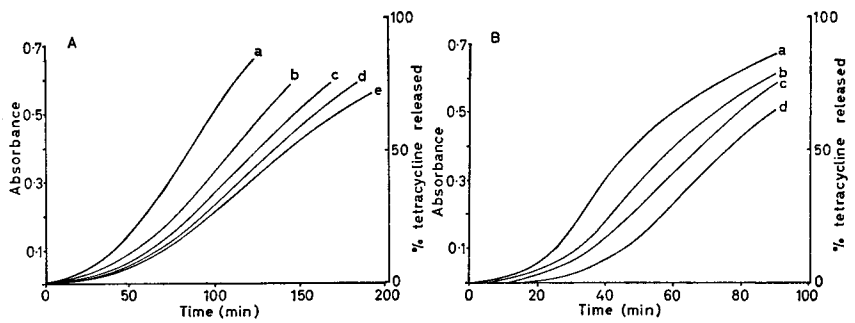

FIG. 2. The release of tetracycline from (A) a film coated 250 mg tetracycline hydrochloride tablet (Cyanamid of Great Britain Ltd.) into 200 ml of (a) water (b) 0·5% (c) 1·0% (d) 1·5% and (e) 2·0% of hog gastric mucin at 37°. Stirring rate 112 rev min^{-1}. Sample size 0·5 ml. Sample frequency 10 min, (B) a sugar coated 250 mg tetracycline hydrochloride tablet. (Thos. Kerfoot and Co. Ltd.) into 200 ml of (a) water (b) 1·0% (c) 2·0% and (d) 3·0% of hog gastric mucin at 37°. Stirring rate 112 rev min^{-1}. Sample size 0·5 ml. Sample frequency 5 min.

to account in part for this apparently anomalous result. The constant as calculated using a mixed solute disc will be influenced by the rate of dissolution of the excipient and the relative amounts of drug and excipient present.

Fig. 2 illustrates the release of tetracycline from two commercially available formulations into dissolution media of varying mucin concentrations. With both, a considerable decrease in release rates were observed on increasing the mucin concentration. This would indicate probable changes in the pharmacokinetics associated with the administration of tetracycline tablets to patients with a history of copious mucus secretion. It is possible that our results may underestimate the true *in vivo* situation,

as the model mucin system possessed rheological properties considerably removed from normal gastric mucus (Janowitz & Hollander, 1954; Curt & Pringle, 1969). This model mucus system is at present being evaluated although it has been accepted as representative by other workers (Barry & Braybrooks, 1974).

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Chemical aspects of penicillin allergy: imidazole-catalysed penicilloylation

We recently established the quantitative relationship of amine structure-reactivity for the penicillin aminolysis (Yamana, Tsuji & others, 1975). The result led to the general rule that, in solutions of neutral pH, amines with a pKa around 7 are the most reactive toward penicillin, whereas amines having a pKa in the region of 10–11, such as ϵ-aminocaproic acid, catalyse the penicilloylation at a very slow rate.

In the understanding of penicillin allergy, the most important problem is how to explain the rapid penicilloylation with the ϵ-amino group of tissue protein under physiological pH and temperature. The following chemical aspects for the *in vivo* formation of penicillin antigen are more acceptable than other possibilities (*e.g.*, see Schneider, 1970); (1) intramolecular rapid penicilloylation *via* combination of the neighbouring functional groups and the ϵ-amino group of lysine (Schneider & de Weck, 1968; Schwartz, 1968, 1969). (2) imidazole-catalysed penicilloylation *via* the highly reactive intermediates of *N*-penicilloylimidazole (Bundgaard, 1972c; Schneider & de Weck, 1974) or its isomerized product, the penicillenic acid (Bundgaard, 1971, 1972a,b). Although a considerable controversy has developed as to which route is important *in vivo*, the decisive pathways are not sufficiently clear.

To explore some possibilities for rapid penicilloylation under physiological conditions, we have examined extensively the reactions of penicillins with various types of imidazole derivatives because these compounds have their pKa values near 7 and can produce more reactive penicilloylamides than other amines. The penicillins we used were benzylpenicillin, ampicillin, cloxacillin and 6-ethoxycarbonylaminopenicillin (ethoxypenicillin) all of which can isomerize to the corresponding penicil-