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THE TRANSPORT OF TETRACYCLINES ACROSS PROTEIN GELS AND SOLS

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The transport of tetracyclines across various model mucus systems has been studied in a three compartment permeability cell. Commercially available hog gastric mucin hydrated in buffer or a buffer containing tetraborate ions to cross-link the gel produced transport rates that were very different from samples of homogenized bronchial mucus. When albumin was substituted for mucus, correlation was obtained for the lag time and the number of moles of tetracycline bound per mole of protein.

INTRODUCTION

If mucus has a protective as well as a lubricant role in the gastrointestinal tract,

then it is probable that the access of drug molecules as well as enzymes and acid to the absorbing mucosa will be restricted by the gel structure. In vitro experiments have demonstrated, however, that for drug molecules which do not complex with the proteins of mucus, for example Warfarin Sodium, the transport rates across such mucus layers are not markedly affected [1]. This no doubt reflects the large water content of mucus gels, which would greatly facilitate drug diffusion. It has been suggested that mucus acts as a molecular filter [2], selectively excluding macromolecules yet providing a free passage for small molecules.

Tetracyclines have been shown to complex with mucus [3] and hence the bioavailability is altered in the presence of mucoidal secretions [4]. The structure of such mucus-tetracycline complexes is unknown, although changes in the viscoelastic properties of mucus on the addition of tetracyclines have been demonstrated [5]. Although tetracycline is capable of permeating mucus, Lawson [6] reported that the quantity of drug diffusing did not decrease linearly with mucin concentration.

It is the object of this work to assess the transport rates of a range of tetracyclines through mucus layers using a variety of 'model mucus' systems. Also included are studies with albumin, a major component of mucus and a protein to which tetracycline binding has been well characterized [7].

MATERIALS

Tetracycline and oxytetracycline (The Boots Co. Ltd) chlortetracycline and demethylchlortetracycline (Cyanamid) and doxycycline (Pfizer Ltd) were used as the hydrochloride salts. The albumin was bovine fraction V (Sigma). The mucus samples were hog gastric mucin (Sigma) dispersed in phosphate buffer (pH 7.0) and allowed to hydrate at 5^{o} for 24 h. The cross-linked gels were prepared by using 0.5 g dl^{-1} disodium tetraborate and adjusting the pH of the dispersion to 8.0 with sodium hydroxide. A pH of 8.0 had previously been shown necessary for tetraborate ions to act as a cross-linking agent [8]. Bronchial mucus was collected from hospitalized patients suffering from a variety of non-tubercular chest conditions. Any contaminated samples were rejected and homogenization achieved by a previously described technique [9].

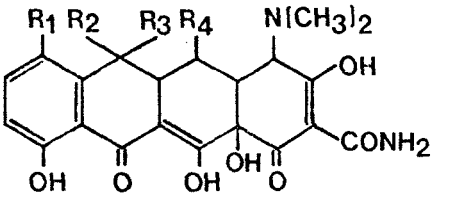
The structure, molecular mass and apparent partition coefficients at 20^{o} for the solvent system 1-octanol/M/15 Sorensens phosphate buffer (pH 7.0) [7] are collated in Table I.

All other reagents were of reagent grade.

METHODS

The Perspex permeability cell consisted of three compartments, 25.4 mm in diameter.

TABLE 1. The Structure, Molecular Mass and Partition Coefficient of the Tetracyclines.



	R_1	R_2	R_3	R_4	M.W.	LnK_{ap}
OT	H	CH_3	OH	OH	496·9	-3·68
T	H	CH_3	OH	H	480·9	-3·30
DMC	Cl	H	OH	H	501·3	-2·80
CT	Cl	CH_3	OH	H	515·4	-1·85
D	H	CH_3	H	OH	512·9	-0·27

The central or protein containing compartment was 3 or 13 mm in length and was separated from the 15 ml donor and receptor compartments by well-soaked Visking membranes. The latter were necessary as the gels were not self-supporting and without such membranes would have dispersed to occupy the total available volume. Both donor and receptor compartments were continuously stirred by underwater magnetic stirrers and the cell was maintained at constant temperature by immersion in a water bath at 37°. The contents of the receptor cell were continuously monitored spectrophotometrically for the tetracyclines. All drugs were dissolved in phosphate buffer (pH 7.0) and controls were carried out with buffer in the central compartment. The cell is shown to scale in Fig. I.

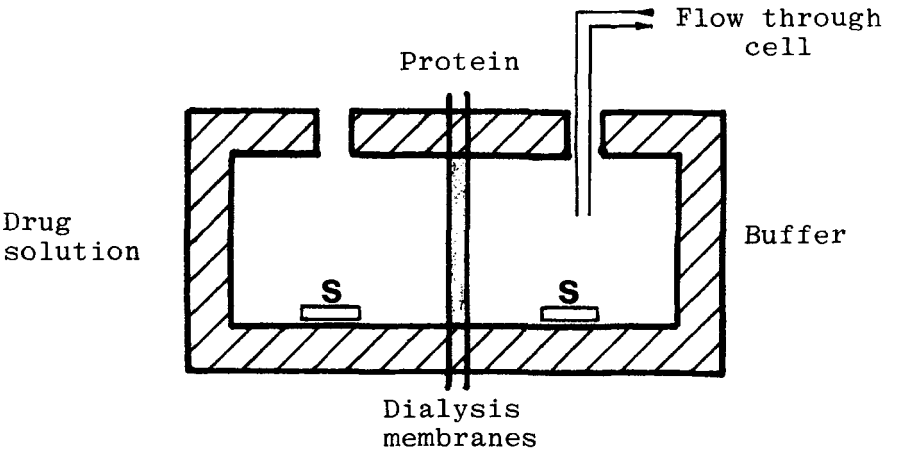


FIGURE 1. The permeability cell. S = magnetic stirers.

The viscosity of hog gastric mucin dispersions was determined by a Contraves Epprecht Rheomat 15 using cup and bob geometry at 37°. The viscosity was calculated from the linear portion of the upcurve of the rheogram.

RESULTS

All the tetracyclines produced experimental curves which could be characterized in terms of a lag time (the time interval before drug appeared in the receptor compartment) and a permeation rate calculated from the slope of a tangent drawn to the permeation curve at an arbitrary but constant time interval.

Representative curves are shown in Fig. 2.

Although the hog gastric mucus samples resulted in a linear relationship between absorbance,

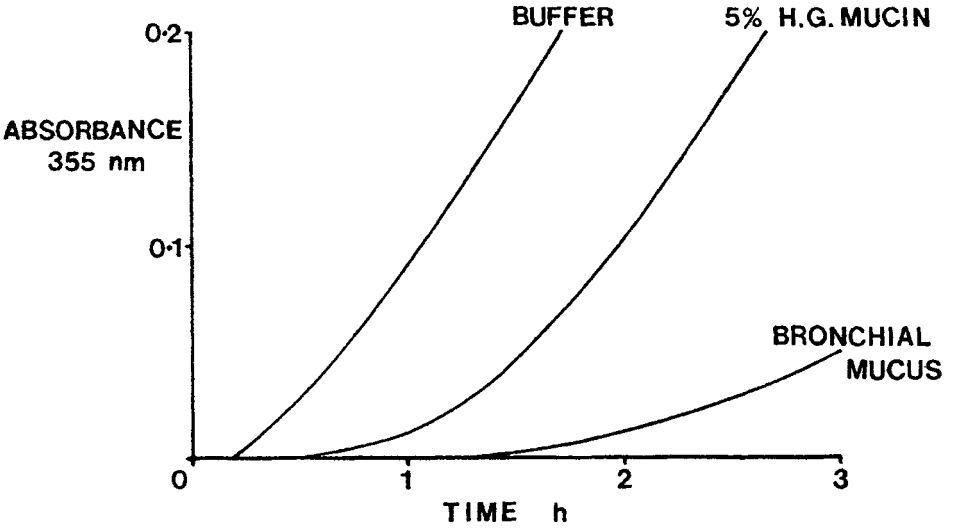


FIGURE 2. Permeation traces for 0.1% tetracycline.

i.e. concentration and time, this was not true for the homogenized bronchial samples. Even after 4 h linearity was not achieved. This may reflect breakdown of the gel network as a result of enzyme action and would, therefore, appear as a continuous increase in permeation rate. It was not, therefore, possible to adopt the Barrer [10] approach to determine lag times, i.e. extrapolation of the linear region to zero concentration from which a diffusion coefficient could be calculated. Also in the absence of a linear region, steady state permeation rates could not be determined, hence the necessity of determining a rate after a set time interval.

Fig. 3 shows the results for the permeation rate of 0.1 g dl^{-1} tetracycline through a 13 mm layer of hog gastric mucin, when a linear

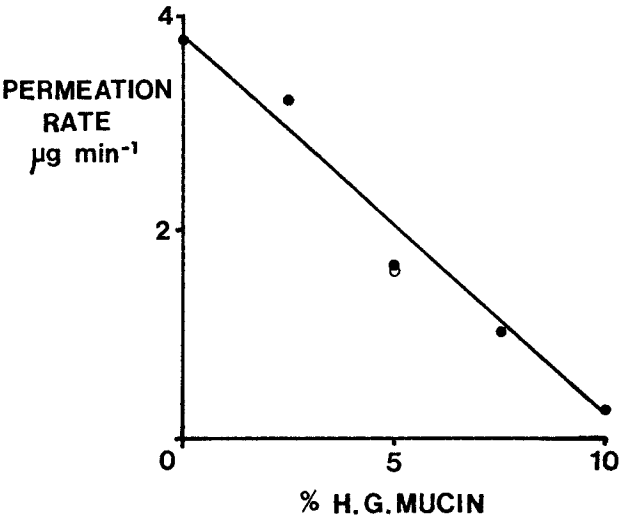


FIGURE 3. Permeation rate as a function of mucin concentration. (O) cross-linked gel.

relationship was observed for permeation rate as a function of mucin content.

Lag times in contrast, yielded a curvilinear relationship (Fig. 4). Measurements were carried out on cross-linked hog-gastric mucin gels at a concentration of 5% mucin.

The cross-linking produced little effect on tetracycline permeation rate (Fig. 3), although an appreciable increase in the lag time resulted. This suggests that cross-linking does not change the amount of available water through which drug molecules diffuse; although binding of the tetracycline molecules to the cross-linked gel network is greater than to the non-crosslinked preparation.

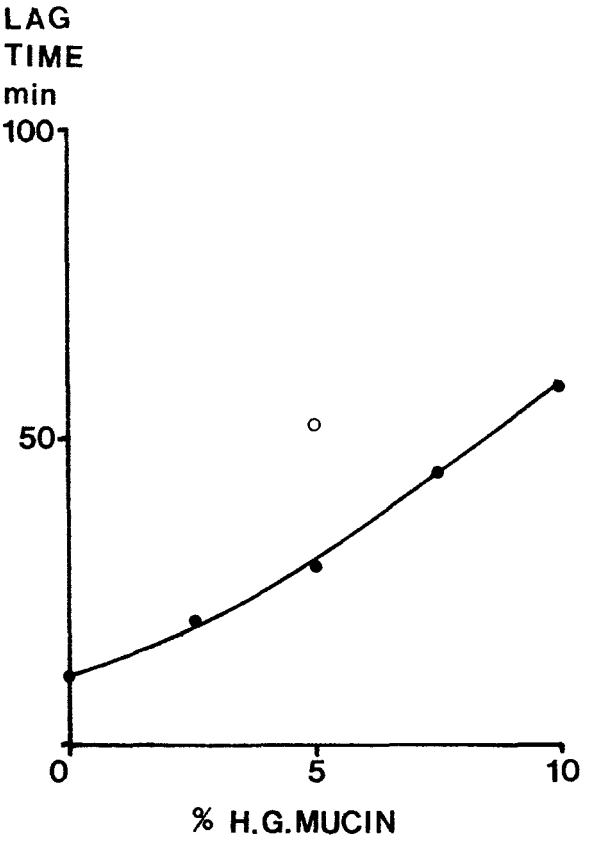


FIGURE 4. Lag time as a function of mucin concentration (O) cross-linked gel.

Linear correlation was not obtained for either viscosity (Fig. 5) or fluidity as a function of the lag time. The failure to show that the lag time was proportional to the viscosity may be due to partial sedimentation of the mucin suspension within the central compartment which would result in a shorter lag time than expected for a homogeneous mucin, and would be greater the higher the mucin content.

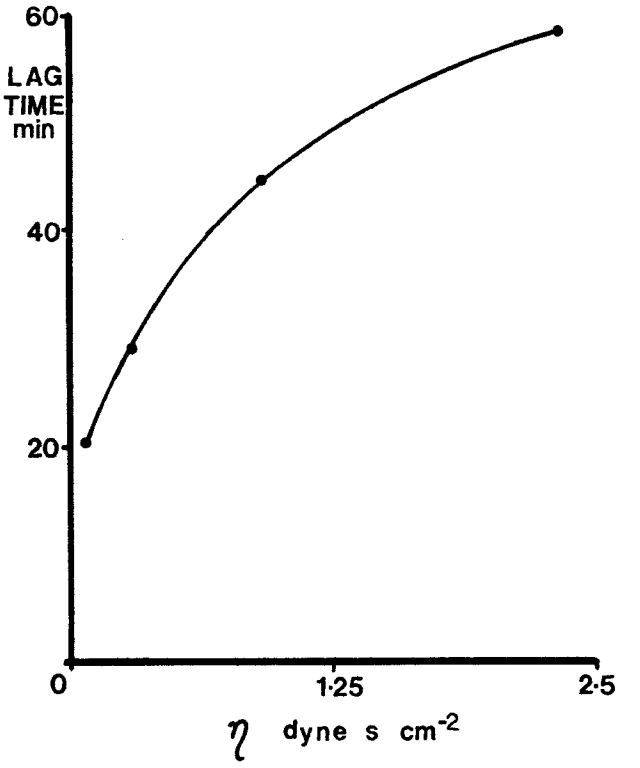


FIGURE 5. The viscosity - lag time relationship.

The lag times for transport of the five tetracyclines through 3 mm layers of homogenized bronchial mucus are represented in Fig. 6. The tetracyclines are presented in order of increasing partition coefficient from left to right. With the exception of chlortetracycline for which a long lag time was recorded, a rank order correlation was obtained. The chlortetracycline may reflect high binding of the drug to the glycoprotein molecules. A preliminary examination, however, of oxytetracycline binding to gastric

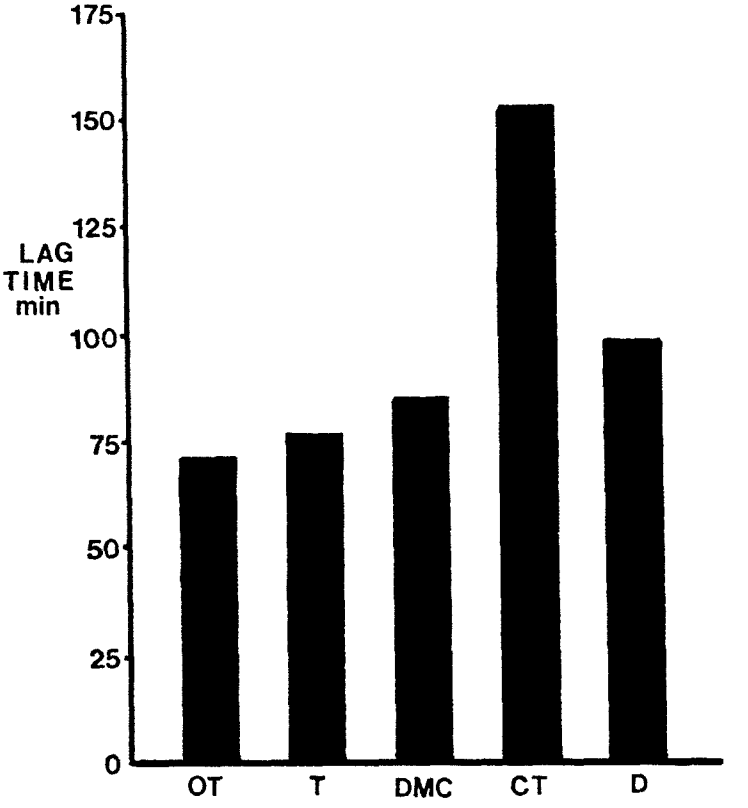


FIGURE 6. The lag times of the tetracyclines through homogenized bronchial mucus.

glycoproteins indicated a low affinity of the drug for the protein [11].

These results may, therefore, reflect the binding of the tetracyclines to other mucus proteins of which albumin is the major component. The experiments were, therefore, repeated with 2% albumin in the central 13 mm compartment. Results (Fig. 7) were expressed as a ratio of the lag time or permeation rate

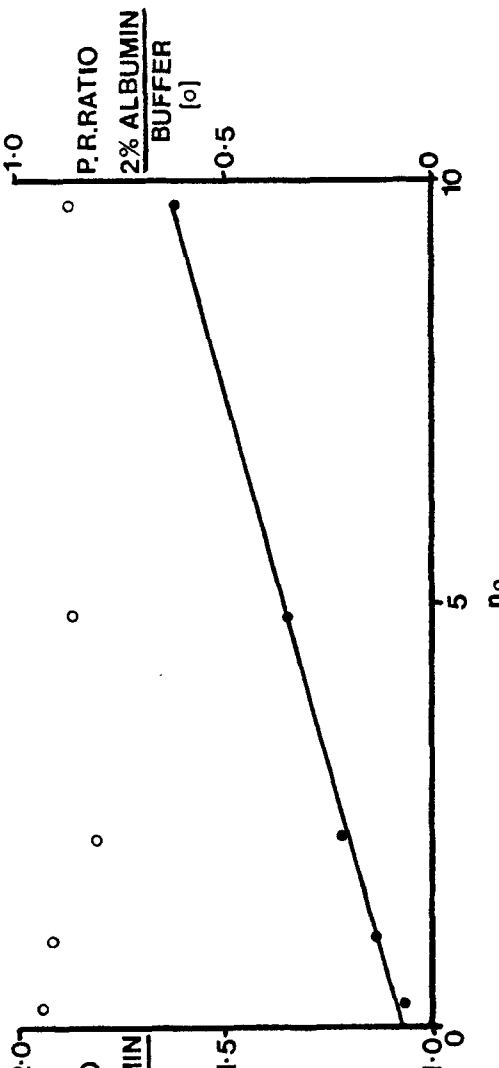


FIGURE 7. Lag time and permeation rate ratios as a function of n_2.

through 2% albumin sol to that through buffer alone. When plotted against the number of moles of drug bound per mole of albumin to the high capacity low affinity site [7], n_2, a significant linear correlation (P=0.95) was obtained for the lag time ratio but not for the permeation ratio. The parameter n_2 was selected because it had previously been shown to be linearly related to log partition coefficient and at a concentration of 0.1% most molecules are bound to the high capacity low affinity site.

Extrapolation of the lag time ratio to zero n_2 yields a ratio of 1.07. This value represents the expected ratio for a tetracycline which is unbound and is greater than unity due to the obstruction effect of the albumin molecules.

In conclusion, mucus layers provide a barrier to the transport of tetracyclines from the lumen to the plasma membrane and may contribute to the variations in peak plasma concentration observed in man [12]. In addition, slow penetration of tetracyclines into bronchial mucus may lead to a delay in the onset of the activity against bacterial infections in the lung.

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