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The effects of mucus glycoproteins on the bioavailability of tetracycline. I. Dissolution rate

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Summary

The effects of a model mucus system on the dissolution rate of tetracycline hydrochloride have been assessed. The model is unique in containing only the rheologically active fraction of the secretion which is a large glycoprotein. A solution of mucus glycoprotein reduced the dissolution rate of tetracycline hydrochloride compared to that in simple acid media although the rate reduction was not the same as that observed in solutions of other viscous agents at the same viscosity. The nature of the results suggests that this occurs as a result of two separate viscosity-related effects. First, the presence of the glycoprotein causes an increased resistance to diffusion but this is less than that expected from the bulk viscosity. In this respect, the mucin resembles other large hydrophillic polymers in having a low, so-called "effective" viscosity. Second, the high viscosity causes a hydrodynamic effect of an increased diffusion layer thickness the maximum value of which is limited by the geometry of the dissolution vessel. Mucus glycoprotein also reduced the dissolution rate and final drug concentration at neutral pH, due to an enhanced build up of crystals of free base on the dissolving solid. This behaviour was altered by the bile salts sodium cholate, deoxycholate and taurodeoxycholate in a manner which can be rationalized in terms of the respective pK_a values.

Introduction

Mucus is a copious secretion of the gastrointestinal (GI) tract where it is thought to act as both a protective and lubricant. There have been several studies into the possible biopharmaceutical consequences of its presence in the tract (Braybrooks et al., 1974; Kellaway and Marriott, 1978; Saggers and Lawson, 1966; Block and Lamy, 1969; Cavallito and O'Dell, 1958; Nimmerfall and Rosenthaler, 1980; Levine et al., 1955), but an aspect which has been largerly overlooked is the effect on dissolution rate. Mucus is present not only as a gel layer on the gastrointestinal epithelium but also as a dilute dispersion in the gastric lumen (Clamp and Brown, 1982). It is reasonable, therefore, to assume that solid dosage forms will disintegrate and dissolve in such an environment. In this work we have investigated the effect of a model mucus system on the dissolution process.

Although mucus is a complex mixture, the component solely responsible for the familiar viscous and gel forming properties is a large glycoprotein or 'mucin' (Snary et al., 1970). In order to avoid any artefactual effects of the other minor components it is desirable that the test medium should

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contain only this rheologically active fraction. Sepharose gel-permeation chromatography has been used successfully to isolate mucus glycoproteins and this technique was selected to purify a commercially available crude powdered form of pig gastric mucin. The pure glycoprotein fraction was then used to assess the effects of mucus glycoprotein on the dissolution rate of a model drug, tetracycline (TC) hydrochloride. Dissolutions were conducted at both acid and neutral pH as a representation of the in vivo extremes. The possible mediation of dissolution behaviour by selected bile salts, namely sodium cholate (SC), deoxycholate (SDS) and taurodeoxycholate (STDC) which coexist in the GI tract was also investigated. For comparison, dissolutions were also performed in solutions of synthetic hydrophillic polymers.

Materials and Methods

The mucus glycoprotein used in this work was that obtained by the purification of a crude dry powdered form (Hog Gastric mucin, Sigma Chemicals, Poole, Dorset). Prior to use the material was "solubilized" using the chaotropic agent potassium thiocyanate (0.22 M in phosphate buffer, pH 7.4) and fractionated on a column of Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden). Only the glycosylated high molecular fraction, as determined by sugar analysis (Winzler, 1955), was retained. This purified gastric mucin (PGM) was concentrated as required using an ultra-filtration cell (Amicon, U.S.A). Biochemical and physical measurements showed the glycoprotein to be a slightly degraded form of the native substance isolated directly from pig stomachs. However, in solution it retained the salient physical feature of the undegraded material in its ability to produce a viscous solution at low concentration.

Tetracycline (TC) hydrochloride, sodium cholate (SC), sodium deoxycholate (SDS), sodium taurodeoxycholate (STDC) and Tris buffer (Tris 7–9) were obtained from Sigma Chemicals, (Poole, Dorset).

Polyvinylpyrrolidone (PVP) 700,000 and 44,000, polyethylene glycol (PEG) 1500 and 4000, and HCl (AnalaR) were supplied by BDH Chemicals (Poole).

[7-^{3}H(N)]Tetracycline was obtained from New England Nuclear, (Boston, MA) and had a specific activity of 37 GBq/mmol.

Dissolution

The drug was prepared as a compressed disc using 200 mg of powder in an evacuable die, 1.3 cm diameter, at a pressure of 9000 kg · cm^{-1}. The disc was fixed into a plastic holder in the base of a 100 ml beaker with the aid of molten paraffin wax such that only the upper surface was exposed. A two-bladed glass stirrer was held centrally by a Perspex former and positioned at a distance of 0.5 cm from the disc surface. The stirrer shaft was coupled to a synchronous motor (Crouzet, Brentford) via a 20 rpm gearbox with a flexible connection to allow for any non-alignment of motor and paddle shafts. The dissolution vessel was immersed in a water bath at 37°C; 40 ml of dissolution medium was added and 0.2 ml aliquots taken every 2.5 min, diluted in 0.1 M HCl and assayed spectrophotometrically at 354 nm (CE 272, Cecil Instruments, Cambridge). Dissolutions were conducted, in duplicate, over a 40-min period and rates were calculated from the linear region of absorbance versus time plots. The solubility of the salt was such that sink conditions were maintained throughout.

0.1 M HCl (pH 1.1) was chosen to simulate gastric pH and solutions were adjusted to be 0.1 M in acid by the addition of concentrated HCl. The dissolution rate in 0.1 M HCl alone was taken as the baseline rate and designated 100% and subsequent rates were compared to this standard. For dissolutions at neutral pH, 0.1 M Tris-HCl, pH 7.4, was used as the medium.

For solubility determinations, excess drug was added to 2 ml of respective medium and the suspension agitated for 6 h at 37°C since previous kinetic studies had shown equilibrium to be achieved within this period. The supernatent was filtered through a 0.2 μm filter (Oxoid, Basingstoke, Hants.) and assayed at 354 nm after appropriate dilution in 0.1 M HCl.

The relative viscosities of solutions was determined using a size A U-tube viscometer at

37°C and corrected to relative dynamic viscosity by multiplying by the relative density. Density measurements were made using a density meter and cell (Parr, Switzerland).

Diffusion studies

Comparative diffusion rates were measured using cylindrical Perspex cells, the central chambers of which were divided by a hydrated Visking membrane into two equal compartments each of 1 ml capacity. The test solution containing polymer additive in 0.1 M HCl was placed in both compartments. Radiolabelled drug diluted in unlabelled drug to a final concentration of 1 mM, was present on one side of the membrane and the initial activity of the labelled drug in this compartment was 12 MBq/ml. The extent of transfer of drug between the compartments was determined by measuring the activity in the receptor compartment after 1 h at 37°C by liquid scintillation. Time course measurements had shown the transfer to be a first-order process and a one point determination of the rate constant was obtained from:

$$k = \ln\frac{(X_0 - 2X_t)}{X_0} \cdot \frac{1}{t}$$

where X_0 is the initial activity in the donor compartment, and X_t the activity in the receptor compartment after time t, in this case, 60 min.

Results

Dissolution in acidic media

With reference to the modified Noyes-Whitney equation:

$$\frac{dC}{dt} = \frac{D \cdot A(C_s - C)}{h \cdot V} \qquad (1)$$

where D = molecular diffusion coefficient; A = surface area; V = dissolution volume; C = instantaneous drug concentration; C_s = equilibrium solubility of drug; h = thickness of the diffusion layer.

Differences in dissolution rate can be expected as a result of a different equilibrium solubility of the drug in the solutions of the additives and this was corrected for using the following expression;

$$R_c = \frac{R_c \cdot C_s}{C_a}$$

where R_c = disolution rate expected from equilibrium solubility; R_c = dissolution rate in acid alone; C_s = equilibrium solubility of TC in the test medium; C_a = equilibrium solubility of TC in acid alone.

In practice, the reduction in solubility was only significant in the PEG and sucrose solutions. The net dissolution rate was expressed as a percentage reduction compared to the rate in acid alone as follows:

$$\%\ \text{Reduction} = \frac{R_c - R_0}{R_c} \cdot 100$$

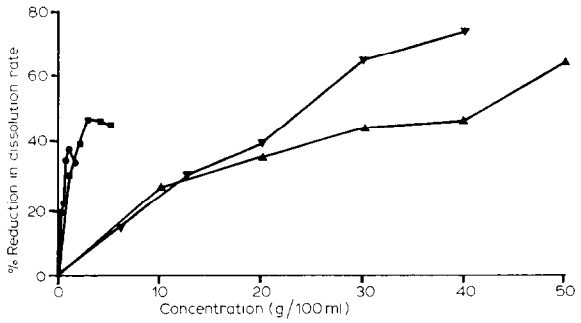

Fig. 1. Percentage reduction in baseline dissolution rate of tetracycline hydrochloride in 0.1 M HCl at 37°C, versus the concentration of various additives. ●, PGM; ■, PVP 700,000; ▼, PEG 1500; ▲, sucrose.

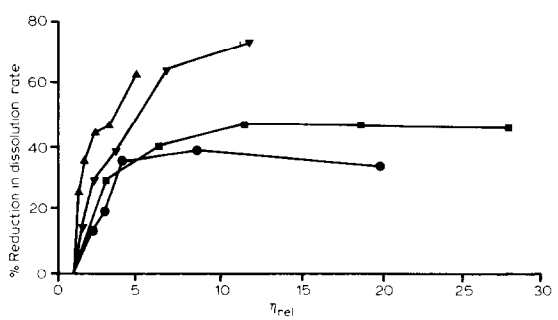

Fig. 2. Percentage reduction in baseline dissolution rate versus relative viscosity for tetracycline hydrochloride in 0.1 M HCl containing various additives. ●, PGM; ■, PVP 700,000; ▼, PEG 1500; ▲, sucrose.

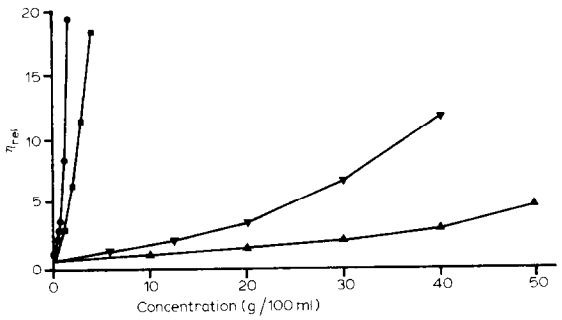

Fig. 3. Relative viscosity versus concentration for solutions of additives in 0.1 M HCl at 37°C. ●, PGM; ■, PVP 700,000; ▼, PEG 1500; ▲, sucrose.

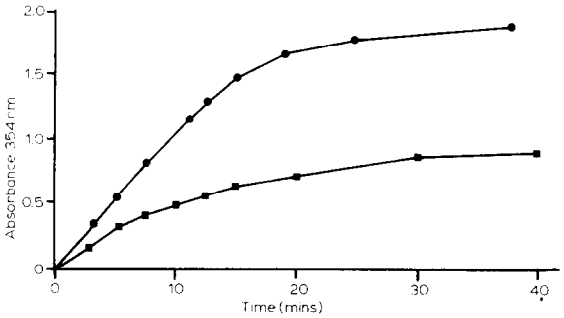

Fig. 4. Dissolution profile showing drug concentration (measured as absorbance at 354 nm) versus time for tetracycline hydrochloride in 0.1 M Tris-HCl, pH 7.4 at 37°C. ■, stirrer speed 20 rpm; ●, stirrer speed 40 rpm.

where R_0 is the actual rate observed.

This percentage reduction for a range of concentrations of the various additives is shown in Fig. 1. Fig. 2 shows the rate reduction versus viscosity for each of the additives while Fig. 3 shows the effect of these additives on the viscosity of the medium. From Fig. 1, on a weight for weight basis, the order of effectiveness in reducing dissolution rate is, PGM > PVP > PEG > sucrose. Also, from Fig. 3, it can be seen that both PGM and PVP impart considerably higher viscosities to the solution than does PEG or sucrose. However, Fig. 2 shows that the rate reduction is not simply related to the increased viscosity, as the reduction at a given viscosity differs widely for each additive. The order of effectiveness in reducing dissolution rate at a given viscosity is thus, sucrose > PEG > PVP > PGM.

Dissolution in neutral media

Fig. 4 shows typical dissolution curves in 0.1 M Tris-HCl, pH 7.4. A high initial rate is observed but this begins to level off after some 5 min and a point is reached where no further dissolution appears to be taking place. At this stage only 30% of the drug disc has dissolved. On inspection, the surface of the disc was seen to be encrusted with large yellow crystals which were considerably larger and more regular than the constituent drug particles of the pellet. A melting point determination gave a value of 160–165°C and this was consistent with the crystals being TC-free base. It is reasonable to assume that the presence of these crystals was preventing complete dissolution of the drug disc.

Fig. 5 shows the effect of 10 mM sodium cholate, deoxycholate and taurodeoxycholate on the dissolution profile. It can be seen that sodium cholate causes a slight reduction in rate while deoxycholate almost completely suppresses dissolution. Taurodeoxycholate, however, does not appear to affect the initial rate but reduces the tendency for the rate to level off. The appearance of a non-crystalline coating on the solid surface was evident with both cholate and deoxycholate while a slight turbidity was observed above the disc when taurodeoxycholate was present.

Fig. 6 shows the dissolution profile when PGM is present in the dissolution medium at a concentration of 1.5% w/v. A substantial rate reduc-

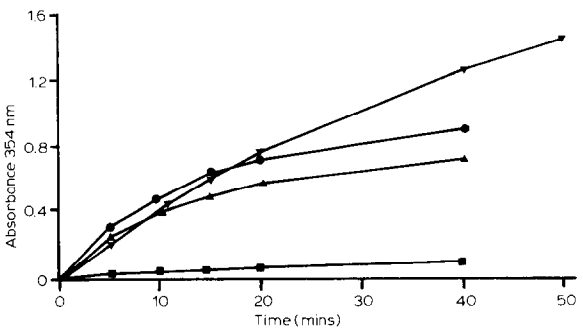

Fig. 5. Dissolution profile of tetracycline hydrochloride in 0.1 M Tris-HCl, pH 7.4, containing various bile salts. ●, control; ▲, 10 mM SC; ■, 10 mM SDC; ▼, 10 mM STDC.

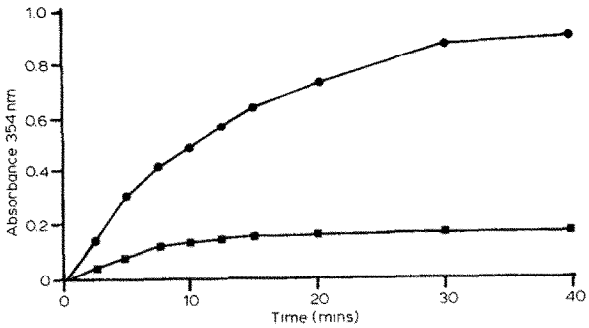

Fig. 6. Dissolution profile of tetracycline hydrochloride in 0.1 M Tris-HCl, pH 7.4 at 37°C, showing drug concentration (measured as absorbance at 354 nm) versus time. ●, control; ■, with PGM at 1.5% w/v.

tion is observed and the final drug concentration reaches no more than 20% of control.

Discussion

Dissolution in acidic media

After correcting for solubility changes, the varying dissolution rates measured for TC-HCl in the different media at the same viscosity must be due to differences in the value of the term D/h of eqn. 1. The diffusion rate is related to viscosity by the Stokes-Einstein equation:

$$D = \frac{kT}{6\pi r\eta} \qquad (2)$$

where k = Boltzmann's constant; T = absolute temperature; r = hydrodynamic radius; η = medium viscosity.

However, small molecules may diffuse faster in some media than others even though the viscosity may be the same (Farng and Nelson, 1973), and this can result in apparently anomalous dissolution behaviour (Florence et al., 1973). It was suggested that this was due to a different diffusional resistance experienced at the molecular level and this was characterized in terms of an "effective" viscosity. In a solution of hydrophillic polymer, although the extended polymer chains impart a high viscosity to the bulk solution, the microenvironment would consist of large areas of free water which would offer little resistance to the diffusing species. The reduction in diffusion rate in such systems would occur, primarily as a consequence of the increased tortuosity of the path taken by the diffusing species. This phenomenon would also appear to apply to solutions of mucus glycoprotein.

The viscosity of the medium will also affect the second component of the D/h term, namely the thickness of the diffusion layer. Developing an equation used by Jost (1960), Nelson (1957) arrived at an expression for the thickness of the diffusion layer when applied to dissolution in a stirred dissolution apparatus;

$$h = (\eta/ds) \qquad (3)$$

where η is the viscosity of the medium in Poise; d the density; and s the speed of the stirrer in revolutions per second. Regardless of the nature of the viscosity-enhancing agent, solutions of similar viscosity will, by definition, have the same hydrodynamic properties because the bulk viscosities are determined from their bulk hydrodynamic behaviour (e.g. U-tube viscometry). This means that unlike the diffusion rate, at a given viscosity, the diffusion layer thickness will be the same irrespective of the type of additive producing the increased viscosity. Consequently, the different dissolution rates measured in different media of the same viscosity can indeed be attributed to differences in diffusivity of the drug in those media. This is confirmed by the comparative diffusion rates for TC (Table 1), which parallels the order of dissolution rate namely, PGM > PVP > PEG > sucrose. Also included in Table 1 are additional grades of the two polymers and it can be seen that there is a molecular weight relation.

On this basis, PGM may be said to exhibit the lowest "effective" viscosity: nevertheless, the reduction in dissolution rate is a substantial one and deserves further consideration. The large rate reduction can be appreciated in terms of the hydrodynamic effect of an increased diffusion layer thickness. With increasing viscosity, the rate drops not only due to the increase in diffusive resistance but also as a consequence of the dissolved drug having to diffuse through an increasingly larger layer of static solvent. That this increase in diffu-

TABLE I

RATE CONSTANTS FOR THE TRANSFER OF TETRACYCLINE IN A TWO-COMPARTMENT CELL IN SOLUTIONS OF VARIOUS ADDITIVES

Additive	Concentration (% w/v) [a]	k [b] (min^{-1}, $\times 10^{3}$)
Control	–	13.0
PGM	1.10	9.6
PVP 700,000	2.34	8.5
PVP 44,000	14.25	5.6
PEG 4000	22.1	5.0
PEG 1500	32.8	3.9

[a] In 0.1 M HCl.
[b] Average of two determinations.

sion layer is a substantial contribution to the reduction in dissolution rate is suggested by Fig. 2. Here, it is seen that for PGM and PVP the percentage reduction rises to a maximum at a relative viscosity of about 10 and this remains constant at higher viscosities. At this viscosity, according to the Nelson equation, the thickness of the diffusion layer will approximate to the distance of stirrer from the surface of the solid (5 mm). It is reasonable to assume that this puts a physical restriction on the size of the layer and hence a further rate reduction, due to an increase in its dimensions, does not occur at higher viscosities.

While it is not suggested that uniform diffusion layers of this thickness are actually present in such a system (the Nelson equation makes some broad assumptions), some limit to the actual dimensions of such a layer might be expected.

Such behaviour, in conjunction with the "effective" viscosity phenomenon, makes the formulation of an equation relating viscosity to dissolution rate extremely difficult. Previous attempts to produce a general dissolution equation have either ignored the effect of a diffusion layer or treated it as remaining constant regardless of the change in bulk viscosity. Usually, an empirical relation is derived with no apparent theoretical basis (Wurster and Taylor, 1965; Sarisuta and Parrot, 1982). A true general equation would require two viscosity terms: one reflecting the viscous drag at the molecular level (the "effective" viscosity) and one describing the bulk viscous flow behaviour of the solvent accounting for the hydrodynamic effect on the diffusion layer. However, it is likely that at high viscosities, due to problems of layer stability, the viscosity–diffusion layer relation is not a simple one and that the diffusion layer thickness, as in this study, reaches some maximum dictated by the scale and geometry of the dissolution vessel. A truely generally applicable dissolution equation is therefore probably indeterminate.

Dissolution in neutral media

The precipitation of free base on the surface of the drug disc during dissolution can be explained in terms of the pH effect on the solubility of weak bases in general. The hydrochloride salt of a weak base such as TC, will dissolve in aqueous media to form the conjugate acid.

$$TC\text{-}HCl \leftrightarrow TCH^{+} + Cl^{-}$$

At acidic pH, the highly soluble protonated form predominates due to the equilibrium:

$$H_3O^{+} + TC \leftrightarrow TCH^{+} + H_2O$$

lying to the right. If the salt is dissolved in a medium of neutral pH, however, the conjugate acid, once in solution, will lose the proton to form the relatively insoluble free base:

$$H_2O + TCH^{+} \leftrightarrow H_3O^{+} + TC$$

If sufficient salt is dissolved initially, enough free base may be formed to produce a supersaturated solution and after a time precipitation will occur. If the dissolution process takes place under conditions of low agitation, such as in the case of the compressed disc, only the diffusion layer will become supersaturated with free base and precipitation will be possible only in this vicinity. In the case of TC-HCl it would appear that crystal nucleation and growth takes place on the surface of the disc itself. Such a process would prevent solvent from reaching the underlying layers of readily soluble salt and further dissolution would be severely restricted. Analagous behaviour has been reported with the sodium salt of the weak acid, tolazamide, where precipitation of free acid reduced the dissolution rate of a compressed pellet

(Higuchi et al., 1965). Also, the deposition of pamoic acid on the pellet surface inhibited the release of benphetamine from benzphetamine-pamoate in acidic media (Morozowich et al., 1962).

The exacerbation of this effect by SDS and SC can be rationalized in terms of the respective pK_a values of these acids. As described above, during dissolution the diffusion layer above the disc will become saturated with TC salt. The pH in the layer may be calculated approximately from (Notari 1975):

$$pH = \tfrac{1}{2}(pK_a - \log C_s) \quad (4)$$

where C_s is the concentration of the salt. The solubility of TC-HCl at 37°C in 0.1 M HCl is 0.33 mol/litre and the acid pK_a is 3.3, hence:

$$pH = \tfrac{1}{2}(3.3 + 4.8) = 1.9$$

The actual pH will be higher than this, albeit only slightly, due to the presence of the Tris buffer whose buffering capacity would be easily exceeded at this salt concentration. The unconjugated bile salts, cholate and deoxycholate, which have pK_a values of around 6 (Small, 1971), are most soluble in the bulk phase of the dissolution medium because at a pH of 7.4 they are present predominantly in the ionized form (96%). In the diffusion layer however, the pH is sufficiently low as to ensure that virtually all the bile acid is in the unionized form. The diffusion layer will now be supersaturated with the free bile acid which will precipitate out and this would explain the deposit on the disc surface. This layer of acid offers additional resistance to drug molecules in the diffusion layer and consequently a lower dissolution rate is measured. The drastic drop in rate caused by SC was probably not due to this effect alone as an increase in medium viscosity was noted when this particular bile salt was used.

Conjugated bile acids, like STDC, have much lower pK_a values of about 1.9 (Small, 1971), hence the situation will be somewhat different when these salts are present. In the relatively acidic environment of the diffusion layer, STDC will still be substantially ionized although significant amounts of free acid will also be present, and this may precipitate out but not to the same extent as the unconjugated acids. This would explain the appearance of merely a slight turbidity over the surface of the dissolving disc. Also, being in the ionized form, STDC would still exert a surface active effect and it may have facilitated dissolution by preventing the deposition of the hydrophobic TC base, probably by raising the solubility.

The importance of the rate of agitation with regard to the precipitation of TC free base, is illustrated by the dissolution profiles observed at neutral pH for two different stirrer speeds (Fig. 4). Doubling the paddle speed to 40 rpm not only increased the initial rate by 60%, but resulted in more than twice as much drug being finally dissolved. The rate would be expected to increase as a consequence of the decreased thickness of the diffusion layer as determined from the Nelson equation. The greater amount of drug finally dissolved arises as a result of a more efficient removal of dissolved free base from the vicinity of the solid surface thus preventing excessive precipitation. It should be pointed out that an apparent dissolution rate in neutral pH which belies the equilibrium solubility of TC-free base ($\simeq$ 1 mg/ml) is a consequence of the drug dissolving in the salt, i.e. protonated form. This protonated form enters solution at a rate determined by its equilibrium solubility which is much higher than that of the free base.

The presence of mucus glycoprotein in the dissolution medium (Fig. 6) will, by virtue of the viscosity enhancing effect, not only reduce diffusion rate but will also increase the depth of the stagnant layer above the dissolving surface. This situation would produce a greater amount of supersaturated solvent in the immediate viscinity of the drug disc, compared to control, and allow a greater degree of base precipitation. The net result would be a reduction in dissolution rate and a decrease in the total amount of drug dissolved. Exactly the same effect could be produced by reducing the stirrer speed (Fig. 4).

In conclusion, a solution of mucus glycoprotein reduces the dissolution rate of tetracycline hydrochloride compared to that in simple aqueous media. This occurs as a result of the higher viscosity which brings about the rate reduction owing to

two separate physical effects. First, there is an increased resistance to diffusion but this is less than expected from the bulk viscosity. In this respect, the mucin resembles other larger hydrophillic polymers in having a low "effective" viscosity. Second, the high viscosity, through a hydrodynamic effect, produces an increase in diffusion layer thickness which reduces the volume of effective mixing. It is probably the latter which is the predominent effect in vivo where conditions of agitation are low. It should be clear that this non-specific effect on dissolution rate will apply to all drug forms.

During dissolution in an environment of neutral pH, precipitation of the base on the solid surface inhibits dissolution and hence the complete dispersal of the underlying TC-HCl. This effect is enhanced in a viscous solution of mucus glycoprotein, probably due to an increased diffusion layer thickness. In vivo, such a situation might arise when the dosage form is prematurely emptied from the stomach into the small intestine. Cholic and deoxycholic acids, which have a low solubilty in the environment of the dissolving drug, further reduce dissolution rate by precipitating on the surface of the solid. This does not occur with the conjugated taurodeoxycholic acid, which appears to reduce the problem of precipitation of the drug itself.

The precipitation of TC-free base during dissolution under low agitation intensity and its exacerbation in a viscous mucus environment, may contribute toward the incomplete absorbtion reported for this drug (Pindell et al., 1959) although the actual significance in vivo remains to be determined.

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