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Effect of protein on the absorption of phenytoin through everted gut preparations

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The transfer of phenytoin across everted gut membrane has been measured both from solutions and from media where the drug is dissolving at different pH. Introduction of protein into the mucosal medium caused varying effects. Protein binding in the mucosal solution causes a decrease in the apparent transfer rate. However, in accord with previous findings, at pH 4 both the solubility and dissolution rate of phenytoin increase in the presence of protein, and this can lead to increased transfer rates. Effects such as these may account for the effect of food on phenytoin bioavailability.

Investigations into the effect of protein on the dissolution of phenytoin showed that both the rate and extent of dissolution could be profoundly affected by small amounts of added protein (Rosen & Macheras 1984). There is a possibility that these effects could cause changes in the absorption of phenytoin, since the in-vivo absorption rate is dissolution rate-controlled. As part of a program towards investigating this possibility we report here on the effects of protein in changing the rate of passage of phenytoin through everted gut preparations.

MATERIALS AND METHODS

Buffer solutions were 0·2 M in phosphate, 0·1 M in citrate, adjusted to give solutions of pH 4, 6 and 7·4. Phenytoin powder (Batch no. 6289, average length 14·6 μm, breadth 8·2 μm) was a gift from Parke Davis and Co. Ltd. Human serum albumin (HSA) 100% electrophoretic purity was from Behringwerke; casein was from Sigma (purified powder).

The gut tissue was from guinea-pigs of either sex, starved for 24 h before death. Sacs of everted intestine 3 cm in length were prepared by standard techniques and contained 0·4 ml of the appropriate buffer. They were placed in 25 ml conical flasks containing 15 ml of pre-oxygenated medium held at 37 °C. In some instances the medium was buffer solution containing 20 μg ml^{-1} of phenytoin with or without a known concentration of protein. In other instances there was no phenytoin present initially, but 5 mg of the powder was added at zero time. The flasks were stoppered and shaken at a frequency of 1 Hz in an incubator held at 37 °C. Sacs were removed for analysis at suitable time intervals for up to 1 h.

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Sacs were washed with the buffer solution and the volume of the contents found by weight. 0·2 ml samples of the contents were then analysed for phenytoin by glc (Macheras & Rosen 1984).

The dissolution of 5 mg samples of phenytoin in buffered solution in the same flasks and under the same conditions as the everted gut experiments was followed to provide profiles of the dissolution occurring.

RESULTS

Transfer across everted gut from phenytoin solutions

In the absence of protein, the total amount of phenytoin entering the sac was never more than 3·5 μg in 1 h. Since the mucosal side of the system contained a total of 300 μg of drug, there was effectively no change in the drug concentration in the mucosal solution. Under these conditions passive absorption would appear to be a zero order process and gives a linear relationship between amount of drug in the serosal compartment mg^{-1} tissue and time.

The specific rate constants for transfer across the gut, calculated using an average value of 0·005 ml mg^{-1} as the volume of a unit amount of tissue, are given in Table 1, which also includes the transfer rates calculated on the basis of the mucosal solution containing 20 μg ml^{-1}.

At the three pH values used, ionization of phenytoin (pK_a 8·3 (Agarwal & Blake 1968)) only becomes appreciable at pH 7·4. Fig. 1 shows that when the specific rate constants are re-calculated on the basis of the concentration of unionized drug present, then there is a linear relation between rate constant and pH.

That passive diffusion of unionized material is

Table 1. Transfer rate constants and transfer rates for the passage of phenytoin through guinea-pig everted gut sacs at 37 °C. Transfer rates refer to: A, transfer from solution; B, limiting transfer from dissolving solid; C, limiting transfer from dissolving solid in presence of 1 mg% HSA.

pH	Specific transfer rate constant ($min^{-1} \times 10^3$)	Transfer rate ($\mu g\ ml^{-1}\ min^{-1}$) A	B	C
4	7·2	0·144	0·147	0·224
6	6·1	0·122	0·126	0·174
7·4	4·4	0·088	0·094	0·094

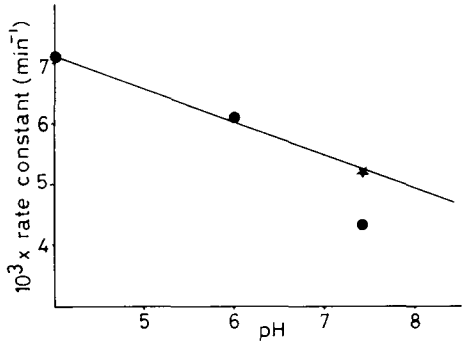

FIG. 1. The effect of pH on rate constant for transfer of phenytoin across guinea-pig everted gut preparations. Mucosal concentration 20 $\mu g\ ml^{-1}$. ●, Specific rate constant calculated using the total mucosal concentration. ★, Specific rate constant calculated using the total unionized mucosal concentration.

occurring was also indicated by two further experiments: (a) at pH 6 the amounts of phenytoin mg^{-1} tissue transported in 1 h from mucosal solutions containing 10, 15 and 20 $\mu g\ ml^{-1}$ were found to be in the ratios 2 : 3·04 : 4·1, and (b) at all three pH values, when phenytoin solutions of 20 $\mu g\ ml^{-1}$ were added to both mucosal and serosal sides of the everted gut, incubation for 1 h resulted in only a slight fall in serosal concentration (ca 10%). This was almost certainly caused by water transport (Pritchard & Porteous 1977), but, whatever the reason, this argues against any uphill transport occurring.

Fingl (1978) has also claimed that phenytoin is absorbed by passive diffusion. An active mechanism was postulated by Johannesson & Strandjord (1975) but this was on the basis of results obtained using very high (500–1000 $\mu g\ ml^{-1}$) concentrations of the drug in solvent at pH 10.

Transfer from solutions containing protein

Experiments in the presence of protein were conducted with HSA (either 0·01 or 1·0 mg ml^{-1}) or with casein (1·0 mg ml^{-1}) in the mucosal compartment. With the drug already in solution before immersion

Table 2. % change in rate of transfer of phenytoin across everted gut membrane in the presence of protein as compared to its absence. Mucosal solutions contained 20 $\mu g\ ml^{-1}$ drug in buffered solution. Values in parentheses are estimates of the % drug bound expected under the conditions of the experiments.

Protein	% of rate in absence of protein pH 4	pH 6	pH 7·4
1 mg% HSA	86 (0)	64 (0)	99 (0)
100 mg% HSA	73 (7·5)	78 (5)	66 (12)
100 mg% casein*	79 —	58 (10)	76 (0)

* Represents amount of casein added, not necessarily that in solution.

of the everted gut sac, at all three pH values used the rate of transfer in the presence of protein was slower than in its absence (Table 2). From other results (Macheras & Rosen, unpublished) it is possible to make estimates of the amount of drug bound under the conditions of these transfer experiments. It can be seen (Table 2) that there is a rough parallel between the estimated amount of protein-bound drug and the fall in the transfer rate. As discussed earlier, this is to be expected since the pseudo zero order rate measured includes the concentration of free phenytoin in its value. At the same time the presence of protein itself would seem to have some retarding effect on the transfer rate, though why this should be so is not apparent.

Dissolution and transfer results with and without added protein

A further set of results was obtained by measuring the rate of transfer across the everted gut membrane using solid drug at time zero rather than a drug solution. In the absence of both protein and the tissue, the rate of dissolution was measured under the same conditions as used for the transfer studies. In all experiments the phenytoin was added as 5 mg of powdered drug. Because such an amount is greatly in excess of the total dissolved at saturation, the particle size is effectively unchanged throughout the dissolution process, which can therefore be taken as first order throughout. There was no difference between dissolution rate constant ($k = 0{\cdot}085\ min^{-1}$) at pH 4 and 6, whilst the rate constant increased at pH 7·4 ($k = 0{\cdot}121\ min^{-1}$). The saturation solubilities at pH 4 and 6 were equal at 30 $\mu g\ ml^{-1}$, while that at pH 7·4 was 42·5 $\mu g\ ml^{-1}$. Bearing in mind the differences in medium, method of agitation, temperature etc. these results are remarkably similar to those reported earlier for the dissolution of phenytoin (Rosen & Macheras 1984).

The effect of protein on the transfer across the

everted gut membrane of phenytoin presented as powdered drug is shown in Figs 2, 3 and 4 for media of pH 4, 6 and 7·4 respectively. Each of these three figures also shows the dissolution profile of the drug, and the transfer of drug across the membrane in the absence of protein when dissolution and transfer are occurring.

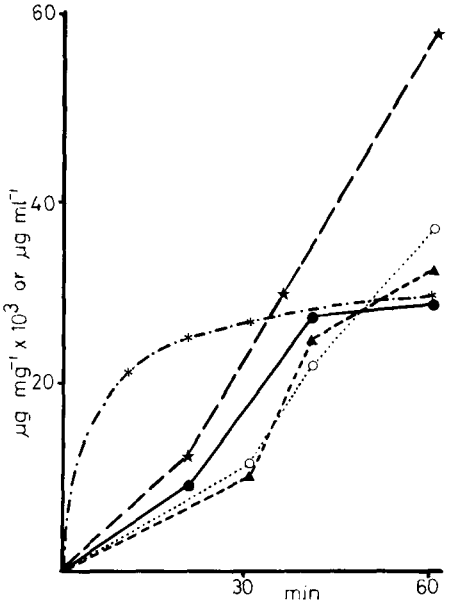

FIG. 2. Amount of phenytoin transferring across guinea-pig everted gut preparations mg^{-1} of tissue at pH 4 as a function of time. 5 mg phenytoin powder was added at zero time. O·····O, buffer solution alone; ★— —★, solution containing 0·01 mg ml^{-1} HSA; ▲– – – –▲, solution containing 1 mg ml^{-1} HSA; ●——●, solution containing 1 mg ml^{-1} casein. The dissolution profile of the powder in buffer alone (in μg ml^{-1}) is also shown ✳—·—·—✳.

In the absence of protein, the fact that the initial mucosal concentration is zero causes a curve in the plot of amount transferred against time, and this curvature tends to be accentuated in the presence of 1 mg ml^{-1} protein.

At low concentrations (0·01 mg ml^{-1}) HSA has a profound effect on the transfer of phenytoin across the everted gut membrane at pH 4 (Fig. 2), the effect being diminished when the pH rises to 6 (Fig. 3) and becoming negligible at pH 7·4 (Fig. 4).

Solutions of 1 mg ml^{-1} HSA have a less significant effect on the transfer. There is some evidence that the initial rate of transfer is enhanced at pH 6, but this has disappeared by the time of the 60 min determination. At all three pHs the amount transferred after 60 min is less than for corresponding experiments in the absence of protein.

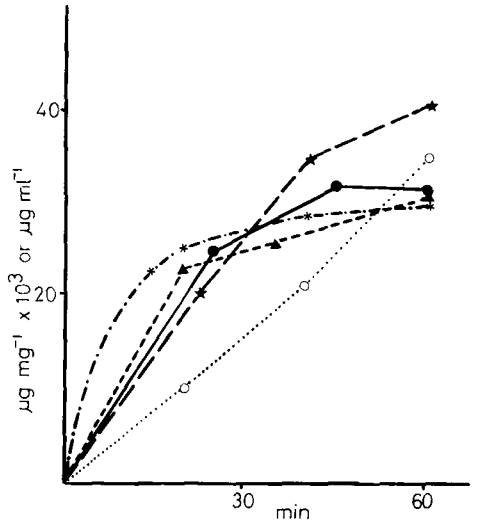

FIG. 3. Amount of phenytoin transferring across guinea-pig everted gut preparations mg^{-1} of tissue at pH 6 as a function of time. 5 mg phenytoin powder was added at zero time. O·····O, buffer solution alone; ★— —★, solution containing 0·01 mg ml^{-1} HSA; ▲– – – –▲, solution containing 1 mg ml^{-1} HSA; ●——●, solution containing 1 mg ml^{-1} casein. The dissolution profile of the powder in buffer alone (in μg ml^{-1}) is also shown ✳—·—·—✳.

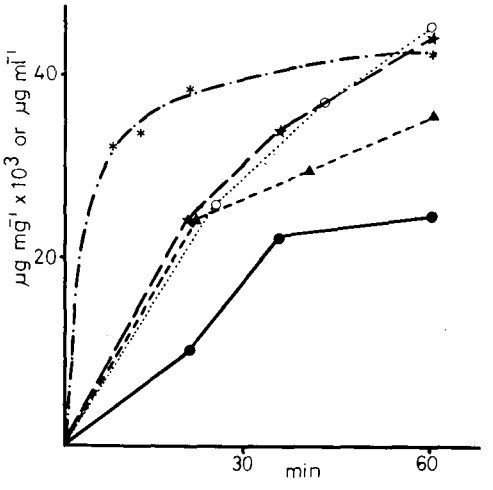

FIG. 4. Amount of phenytoin transferring across guinea-pig everted gut preparations mg^{-1} of tissue at pH 7·4 as a function of time. 5 mg phenytoin powder was added at zero time. O·····O, buffer solution alone; ★— —★, solution containing 0·01 mg ml^{-1} HSA; ▲– – – –▲, solution containing 1 mg ml^{-1} HSA; ●——●, solution containing 1 mg ml^{-1} casein. The dissolution profile of the powder in buffer alone (in μg ml^{-1}) is also shown ✳—·—·—✳.

Casein at 1 mg ml^{-1} behaves rather like 1 mg ml^{-1} solutions of HSA at pH 4 and 6, but causes a noticeable reduction in transfer rate at pH 7·4, with the amount of phenytoin crossing the membrane

almost halved throughout the time course of the experiment.

DISCUSSION

In considering the results depicted in Figs 2–4 it must be remembered that the transfer rates shown are the results of two processes, that of dissolution and that of transfer itself. The general equation for the system is

$$C_T = C_s\{1 - [(K e^{-kt} - k e^{-Kt})/(K - k)]\} \quad (1)$$

where C_T, C_s are the concentration in the serosal compartment and the saturating concentration in the mucosal compartment respectively, and K, k are the rate constants for dissolution and transfer.

The presence of protein can affect the rate in a number of ways. Results for transport from solution show (Table 2) that the proteins affect the rate by binding the drug and possibly by some other mechanism, which might be a change in viscosity of the medium. At the same time it is known (Rosen & Macheras 1984) that proteins can affect both the rate and extent of dissolution of phenytoin. Qualitatively the transfer results agree with these two influences. Thus at pH 4 and with 1 mg ml^{-1} HSA, a predicted increase in dissolution rate in the presence of protein is balanced by a predicted decrease in transfer rate because of protein binding. As time increases, only the slower (transfer) rate is rate-determining so that the amount transferred in the presence of these large amounts of protein is at first equal or greater than that in the absence of protein, but then drops below.

At pH 7·4 and at protein concentrations of from 0·02–0·12 mg ml^{-1} the dissolution rate of phenytoin in the presence of casein is reduced by about a third (Rosen & Macheras 1984). If the same tendency holds for casein concentrations of 1 mg ml^{-1}, then this, coupled with the depressing effect of casein on the transfer rate from solution (Table 2), accounts for the low transfer in the presence of casein seen in Fig. 4.

Results in the presence of only 0·01 mg ml^{-1} HSA, or in the absence of protein altogether, are more amenable to quantitative analysis, since the uncertainties of extrapolated binding results are no longer present. So far as transfer in the absence of protein is concerned, the fact that the initial mucosal concentration of phenytoin is zero causes a curve in the line. However, at all pH values, dissolution is almost complete after 30 min, so that the terminal part of the lines can be used to estimate the rates of drug transfer near the point of maximum solubility. These estimates are given in Table 1. A similar analysis of the terminal portions of the curves of Figs 2–4 was made to determine transfer rates in the presence of 0·01 mg ml^{-1} HSA (Table 1). HSA, 0·01 mg ml^{-1} (=1 mg%), is within the range of HSA concentrations previously investigated for their effect on phenytoin solubility and dissolution rate (Rosen & Macheras 1984). From the latter results, predictions of the solubilities in the presence of the protein at pH 4 and pH 7·4 can be made as 45 and 44 μg ml^{-1} respectively. These compare with values calculated from the ratios of the transfer rates (Table 1, columns B and C) together with the solubilities in absence of protein which give values of 45·7 and 42·5 μg ml^{-1}. There is a good agreement between calculated and predicted solubilities.

With known dissolution and transfer rate constants, and known saturation solubility, equation (1) can be used to predict the amount of drug transferred with time. Table 3 compares the results for the amount of transfer found in the presence of 1 mg% protein at pH 4 after various times with those predicted on the basis of the dissolution rate constant found in the absence of protein. The calculation is repeated for a rate constant almost twice this value, since it was previously shown (Rosen & Macheras 1984) that the dissolution rate of phenytoin at pH 4 is about doubled in 1 mg% HSA solution. The greater correspondence between experimental and calculated values in the latter case again shows that the results of the dissolution work previously reported extend to the transfer stage of absorption.

Table 3. Amounts of phenytoin transferred at pH 4 in the presence of 0·01 mg ml^{-1} HSA compared with values predicted from equation (1).

Time (min)	Amount transferred (μg mg^{-1} tissue) Found	Calculated[1]	Calculated[2]
20	11·5	11·2	15·1
35	30·0	25·0	30·0
60	58·0	47·9	53·0

[1] Dissolution rate constant taken as 0·085 min^{-1}.
[2] Dissolution rate constant taken as 0·16 min^{-1}.

Corrigan et al (1980) have shown that there is enhanced transport across an artificial membrane when sulphathiazole—PVP or hydrocortisone—PVP co-precipitated solutions are used rather than solutions of the drug by itself. Similarly O'Driscoll & Corrigan (1981) have found enhanced transfer of chlorothiazide—PVP co-precipitate from solution across the everted gut.

The present work has concentrated more on transfer from a system in which drug is dissolving, rather than solution itself. The results are in good agreement with previous studies of the effects of protein on the dissolution of phenytoin and show that these effects are likely to affect the transport process in the following ways:

(i) alter the dissolution rate and hence the rate at which drug is made available for transfer.

(ii) alter the saturation solubility and hence the concentration gradient which controls the rate of transfer.

(iii) alter the concentration of free drug in solution and therefore, again, the concentration gradient which controls the rate of transfer.

(iv) alter the physicochemical properties of the system (viscosity, porosity, extent of aggregation, etc.).

All such possible alterations are, in turn, dependent on pH, the nature of the protein and its concentration, the first two of these, and possibly all three, being inter-related. At 0·01 mg ml^{-1} the molar concentration of HSA is about one thousandth that of a saturated phenytoin solution, so that these alterations can become apparent at surprisingly low protein concentrations.

Effects such as those summarized above are likely only to be important for drugs which, like phenytoin, are intrinsically sparingly soluble. They may account for the effect of food on phenytoin absorption (Melander et al 1979). They add to the known complexity of the effects of food on bioavailability (Melander 1978) and provide yet a further reason for the difficulties encountered in providing dissolution tests which give better than rank order correlations with in-vivo results.

REFERENCES

Agarwal, S. P., Blake, M. I. (1968) J. Pharm. Sci. 57: 1434–1435

Corrigan, O. I., Farvar, M. A., Higuchi, W. I. (1980) Int. J. Pharm. 5: 229–238

Fingl, E. (1978) in: Pippenger, C. E., Penry, J. K., Kutt, H. (eds) Antiepileptic Drugs: Quantitative Analysis and Interpretation, Raven Press, NY, p 221

Johannesson, S. J., Strandjord, R. E. (1975) in: Schneider, H. (ed.) Clinical Pharmacology of Antiepileptic Drugs, Springer-Verlag, Berlin, p 262

Macheras, P., Rosen, A. (1984) Die Pharmazie 19: 322–325

Melander, A. (1978) Clin Pharmacokin. 3: 337–351

Melander, A., Brante, G., Johansen, O., Lindberg, T. (1979) Eur. J. Clin. Pharmacol. 15: 269–274

O'Driscoll, K. M., Corrigan, O. I. (1981) in: Aiache, J. M., Hirtz, J. (eds) Proc. 1st Eur. Cong. Biopharm. Pharmacokin. Technique et Documentation, Paris, p 71

Pritchard, P., Porteous, J. (1977) Biochem. J. 164: 1–14

Rosen, A., Macheras, P. (1984) J. Pharm. Pharmacol. 36: 723–727