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An in vitro model for assessing drug availability from lipophilic vehicles

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An apparatus for studying the rate of release of a solute from a water-immiscible solvent into an acidic, aqueous liquid, followed by permeation through a simulated lipid membrane, is described. The object was to imitate the absorption of a drug after oral administration in a soft gelatin capsule. Rate constants of transfer were determined for a series of substituted benzoic acids, using octanol and isopropyl myristate as solvents. The quantity of solute in the acidic phase did not correlate with the solvent-water distribution coefficient, but was linearly related to the rate constant for transfer from solvent to water. A dynamic system was therefore postulated, rather than one in which the two phases are in equilibrium. In vivo studies on two of the solutes confirmed the in vitro observations. No simple relationship could be derived between blood concentrations and any in vitro parameter, but the rank order of magnitude of the blood concentrations fitted the postulated dynamic mechanism.

Whilst drug dissolution from solid dosage forms has been extensively studied, the development of methodologies for the assessment of drug release characteristics from lipid-containing dosage forms has been neglected in comparison. It was therefore the aim of this investigation to develop methods by which the release of drugs from solution in lipophilic solvents could be assessed in vitro. It has been established (Muranishi et al 1971) that the mechanism of drug absorption in vivo from lipid solution involves the liberation of the drug from the vehicle into the aqueous luminal fluid, followed by absorption through the gastrointestinal wall. Direct absorption from the oil phase can be neglected. Accordingly a multicompartmental apparatus, which simulates diffusion of a drug from a non-aqueous solution into the gastric contents, and thence through a membrane into the circulation, has been designed.

MATERIALS AND METHODS

Materials

The solutes investigated were 4-hydroxy and 4-alkoxybenzoic and phenylacetic acids, and are listed in Table 1. Their purities, together with those of the non-aqueous solvents (1-octanol and isopropyl myristate) and relevant physicochemical data, are described elsewhere (Armstrong et al 1979).

The two radioactive acids used in this study, 4-hydroxybenzoic acid [carboxyl-^{14}C] and 4-methoxybenzoic acid [carboxyl-^{14}C], possessing specific activities of 55 and 50 mCi mM^{-1} respectively were obtained with 99% purity from Centre d'Etudes Nucleaires de Saclay.

Apparatus

The partition-permeation apparatus, shown in Fig. 1, consisted of two identical rectangular Perspex blocks, each bored out to form a cylindrical cell, 70 mm in diameter and 130 mm high, giving a maximum capacity of 400 ml. Cell contents were agitated by two twin-3-bladed stainless steel stirrers, each mounted on a shaft running down the axis of the cylinder, and driven at 30 rev min^{-1} by a synchronous electric motor. The contents of the cylinders, when clamped together, were connected by a circular hole of area 491 mm^2 bored through the sides of the blocks near their base. A Sartorius cellulose nitrate filter, impregnated with a simulated gastric lipoid barrier (Stricker 1971, V. A. Howe, London), was fixed between the blocks at this point, so as to separate the contents of the two cylinders.

The apparatus was immersed in a water bath at 37 °C. 150 ml of pH 1·2 buffer, previously equilibrated with the appropriate non-aqueous solvent, was pipetted into one cylinder and 300 ml of pH 7·4 buffer into the other. 150 ml of a solution of the solute under investigation in the appropriate non-aqueous solvent was carefully pipetted onto the low pH solution. Both aqueous solutions were stirred for 8 h, and monitored in turn by passing peristaltically along PTFE tubing through a flow cell, mounted in a u.v. spectrophotometer (Cecil Instruments Ltd., Model CE272). The stirrer blades were angled so that no disruption of the solvent-water interface

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Table 1. Partitioning and permeation rate constants and distribution coefficients for a series of substituted benzoic acids.

Acid	Solute No	Lipophilic solvent: Octanol k_{AB}	k_{BA}	k_{BC}	$\frac{k_{BA}}{k_{AB}}$	Dist. coeff.	Isopropyl myristate k_{AB}	k_{BA}	k_{BC}	$\frac{k_{BA}}{k_{AB}}$	Dist. coeff.
4-Hydroxybenzoic	1	0·0595	1·53	0·0420	25·6	25·6	0·108	0·0610	0·0300	0·565	0·545
4-Methoxybenzoic	2	0·0173	1·36	0·0605	78·4	76·0	0·0510	0·448	0·0650	8·78	8·72
4-Ethoxybenzoic	3	0·0059	1·50	0·0840	255	243	0·0220	0·629	0·0750	28·6	28·1
4-Hydroxyphenyl acetic	4	0·243	1·32	0·0100	5·44	5·65	0·110	0·0170	0·0080	0·155	0·127
4-Methoxyphenyl acetic	5	0·0510	1·47	0·0430	28·8	29·9	0·0820	0·260	0·0400	3·17	2·95
4-Ethoxyphenyl acetic	6	0·0160	1·34	0·0600	83·9	89·7	0·0480	0·490	0·0600	10·2	9·60
4-Hydroxyphenyl propionic	7	0·0790	1·21	0·0220	15·3	14·4	0·109	0·0520	0·0170	0·477	0·439
4-Methoxyphenyl propionic	8	0·0182	1·46	0·0650	80·2	79·0	0·0440	0·470	0·0650	10·7	10·9
4-Methoxyphenyl butyric	9	0·0065	1·43	0·0730	219	214	0·0185	0·564	0·0750	30·5	27·3

occurred, and the volume of liquid involved in the sampling circuits did not exceed 4·5 ml. Spectrophotometer output was recorded on chart (Servoscribe potentiometric recorder, Smiths Industries Ltd.).

In vivo methodology

Male Wistar rats, 260 to 280 g, fasted for 20 h, but allowed free access to water, were anaesthetized by intraperitoneal injection of urethane and the stomach was exposed by midline incision. The oesophagus was ligated immediately adjacent to the cardiac sphincter, and the stomach contents squeezed gently into the duodenum, which was then ligated at the pyloric sphincter.

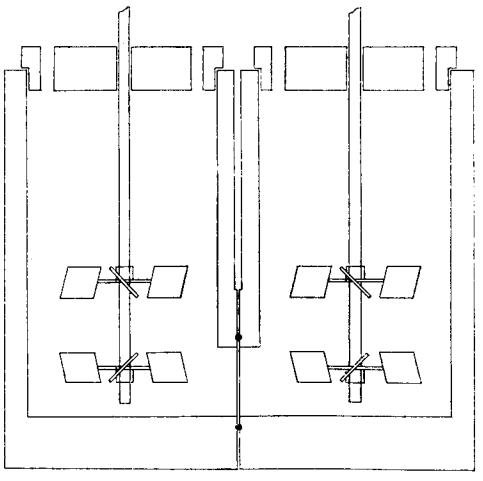

FIG. 1. A cross-sectional view of the partitioning-permeation cell.

The labelled benzoic acid derivatives were dissolved in either octanol or isopropyl myristate, and diluted with the appropriate non-radioactive compound to give a specific activity of 2·5 μCi mg^{-1}. Three ml of isotonic saline adjusted to pH 1·2 with hydrochloric acid were injected into the stomach, followed by 0·5 ml of drug in the non-aqueous solvent so that each rat received a total of 1·0 mg of substituted benzoic acid and a total activity of 2·5 μCi.

After fixed time intervals, the stomachs were isolated and the aqueous and non-aqueous phases of the gastric contents sampled. Blood samples were obtained by cardiac puncture, and each experiment was carried out in quadruplicate. Radioactivity was measured by liquid scintillation counting (Beckman Model LS-235), the concentration of solute in the stomach being corrected for water flux by the method of Schanker et al (1957). The scintillation cocktail of Hall & Cocking (1965) was used.

RESULTS AND DISCUSSION

The concentrations of diffusant in the two aqueous phases in vitro were calculated from experimental absorbance data. Preliminary experiments showed that for the compounds being investigated, backward transfer from the solution of high pH to that of low pH was negligible, due to the almost total ionization of the compounds at pH 7·4. Furthermore, the lipid-impregnated membrane, which might be expected to constitute an operative fourth phase, was found to

retain negligible amounts of diffusant, so that it could be assumed that the concentration gradient within the membrane was instantaneously adjusted to external conditions. This also enabled the concentration remaining in the non-aqueous phase to be calculated by a mass balance relationship, the total amount of drug present and the concentrations in the two aqueous phases being known.

A simultaneous partition-permeation process of the type described above can be represented diagrammatically by Fig. 2, in which compartments A, B and C are the non-aqueous, aqueous pH 1·2 and aqueous pH 7·4 phases respectively. Specimen sets of results are plotted in Figs 3 and 4, and are typical of the model postulated. Concentrations are presented as the fraction of the total drug remaining in the compartment, thus F_B represents $[B]/[A_o]$, where $[A_o]$ is the initial concentration of drug in compartment A and [B] is the concentration in compartment B at the time of interest. Each point on the graph is the mean of at least two determinations.

Since backward transfer from compartment C to B, and membrane retention are negligible, the kinetics of the system can be described by equations 1 to 3, where [A], [B] and [C] are the concentrations of drug in the respective compartments and k_{AB}, k_{BA} and k_{BC} represent transfer rate constants.

$$\frac{d[A]}{dt} = -k_{AB}[A] + k_{BA}[B] \quad .. \quad .. \quad (1)$$

$$\frac{d[B]}{dt} = k_{AB}[A] - k_{BA}[B] - k_{BC}[B] \quad .. \quad (2)$$

$$\frac{d[C]}{dt} = k_{BC}[B] \quad .. \quad .. \quad .. \quad (3)$$

Rescigno & Segre (1966) have shown that in the closed three compartment system illustrated in Fig. 2 F_B is related to time through equation 4. X_1 and X_2

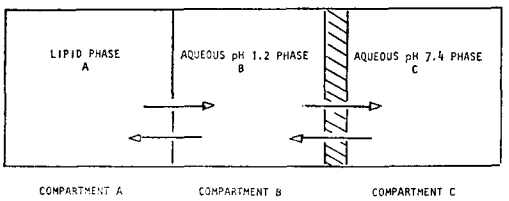

FIG. 2. The partitioning-permeation model. Compartment A: $[A]_{t=0.} = A_o$. Compartment B: $[B]_{t=0} = 0$. Compartment C $[C]_{t=0} = 0$.

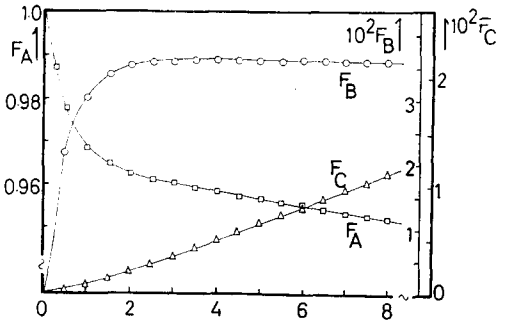

FIG. 3. Plots of the fractions of 4-hydroxybenzoic acid in the n-octanol compartment (F_A), the aqueous pH 1·20 compartment (F_B) and the aqueous pH 7·40 compartment (F_C) as a function of time. Ordinate: fraction. Abscissa: time (h).

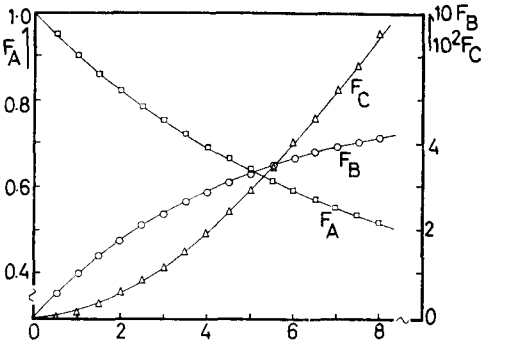

FIG. 4. Plots of the fractions of 4-hydroxybenzoic acid in the isopropyl myristate compartment (F_A), the aqueous pH 1·20 compartment (F_B) and the aqueous pH 7·40 compartment (F_C) as a function of time. Ordinate: fraction. Abscissa: time (h).

are constants characteristic of the system, and a and b are defined by equations 5 and 6. The rate constants of the kinetic model expressed by equations 1 to 3 were calculated from equations 7 to 9, using these constants (Doluisio et al 1970). The coefficients of equations 4 were determined by applying the maximum likelihood curve fitting program (MLP). In some cases, when the exponential coefficient approached zero, the program failed to fit the data to a biexponential equation, and the graphical method of 'feathering' had to be used (Wagner 1975). These initial estimates were refined using the continuous system modelling program (CSMP) of McArthur (1972).

$$F_B = X_1e^{-at} + X_2e^{-bt} \quad .. \quad .. \quad .. \quad (4)$$

$$a = \tfrac{1}{2}(k_{AB} + k_{BA} + k_{BC}) - \sqrt{(k_{AB} + k_{BA} + k_{BC})^2 - 4k_{BA}k_{AC}} \quad (5)$$

$$b = \tfrac{1}{2}(k_{AB} + k_{BA} + k_{BC}) + \sqrt{(k_{AB} + k_{BA} + k_{BC})^2 - 4k_{BA}k_{AC}} \quad (6)$$

$$k_{AB} = \frac{X_1a + X_2b}{X_1 + X_2} \quad .. \quad .. \quad .. \quad (7)$$

$$k_{BC} = \frac{ab}{k_{AB}} \quad .. \quad .. \quad .. \quad (8)$$

$$k_{BA} = \frac{a + b}{6} - k_{AB} - k_{BC} \quad .. \quad .. \quad (9)$$

The values for the rate constants k_{AB}, k_{BA} and k_{BC} are given in Table 1. The ratios of k_{AB} to k_{BA} are in good agreement with the distribution coefficients, determined in the traditional manner (Armstrong et al 1979).

The concentration in compartment B, shown in Fig. 3, reached a steady state within 3·5 h of the start of the experiment. It could therefore be assumed that compartments A and B were in equilibrium. Under these circumstances, equation 1 simplifies to equation 10, where K represents the equilibrium constant between the two phases. Furthermore, since only about 1% of the hydroxybenzoic acid in the octanol phase was consumed, F_A can be considered constant, and F_B becomes directly proportional to K. All the graphs, except those obtained with the phenolic acids in the isopropyl myristate system, followed the same pattern as Fig. 3 but regression analysis of the experimental results did not produce the anticipated rectilinear relationship between F_B and K. Studies on rats confirmed this conclusion, though, as only two labelled compounds were examined, data derived from this source must be evaluated with caution. Maximum concentrations of carbon-14 in the aqueous phase of the gastric contents are shown in Table 2, and follow the same rank order as the in vitro distribution coefficients. However, there is no simple relationship between the two parameters.

$$F_B = KF_A \quad .. \quad .. \quad .. \quad (10)$$

$$F_B = \frac{k_{AB}}{(a - b)}(e^{-bt} - e^{-at}) \quad .. \quad .. \quad (11)$$

Rescigno & Segre (1966) proposed equation 11 for evaluating F_B in the kinetic model expressed by equations 1 to 3. This suggests that F_B is mainly dependent on k_{AB}, the influence of k_{BA} and k_{BC} being confined to their contributions to a and b (see eqns 5 & 6). Regression analysis of the steady state values of F_B for the 15 systems which followed the same pattern as that exemplified by Fig. 3, yielded a good rectilinear relationship ($r = 0{\cdot}950$, $n = 15$), confirming that F_B was almost exclusively dependent on k_{AB}. A rectilinear relationship ($n = 0{\cdot}944$, $n = 4$) was also obtained between maximum concentrations of drug in the aqueous phase of the gastric contents in vivo, and the in vitro k_{AB} values. The in vivo results therefore support the conclusion that

Table 2. In vivo absorption data for a series of substituted benzoic acids. I Maximum aqueous phase concentration in gut.[a] II Quantity absorbed from gut after 4 h.[a] III Maximum blood concentration (g ml^{-1}).

Solvent	Solute	I	II	III
Octanol	4-Hydroxy benzoic acid	11	28	0·6
Octanol	4-Methoxy benzoic acid	4	41	1·1
Isopropyl myristate	4-Hydroxy benzoic acid	50	61	1·5
Isopropyl myristate	4-Methoxy benzoic acid	15	90	2·4

[a] Percentage of dose administered.

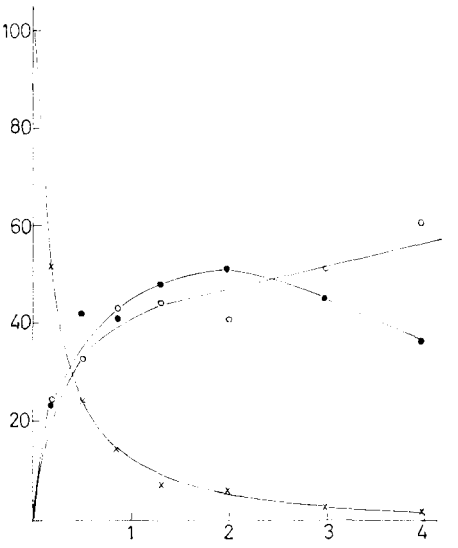

FIG. 5. Distribution profile of 4-hydroxybenzoic acid administered as a solution in isopropyl myristate. × : isopropyl myristate phase. ●: gastric aqueous phase. ○: absorbed from gut. Abscissa: time (h). Ordinate: % initial dose.

aqueous concentrations of drug in the gut are dependent on rate of transfer, rather than distribution coefficient.

In the isopropyl myristate system in vitro, none of the phenolic acids reached a steady state in the acidic buffer phase over the time studied. Fig. 4 is an example. Only one of these combinations was examined in vivo and this also gave aqueous gastric concentrations which passed through a maximum with time (Fig. 5). The other three combinations examined in vivo, of which Fig. 6 is an example, gave concentrations which after an initial sharp rise increased only marginally with time, a similar behaviour to that observed in vitro. These isopropyl myristate systems which gave a steady state build up of drug in compartment B, gave higher values of F_B than the corresponding octanol systems. Similarly, the fraction which appeared in compartment C (F_C) after a given time, was always higher for the isopropyl myristate system than for the octanol system. The inference is therefore that solutions in isopropyl myristate release their solutes into the gut more rapidly than those in octanol, and this is borne out in a limited manner by the biological results. The quantity of drug absorbed after a given time can be

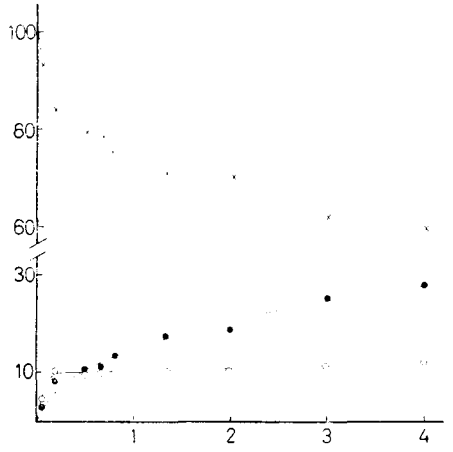

FIG. 6. Distribution profile of 4-hydroxybenzoic acid administered as an n-octanol solution. × : n-octanol phase. ○ : gastric aqueous phase. ● : absorbed from gut. Ordinate: % of initial dose. Abscissa: time (h).

calculated by subtracting the total drug found in the gut from that originally administered. Percentages absorbed after 4 h are given in Table 2, and show that in vivo 4-hydroxybenzoic acid and 4-methoxybenzoic acid are absorbed more rapidly from isopropyl myristate than from octanol. Blood concentrations obtained after administration of 4-

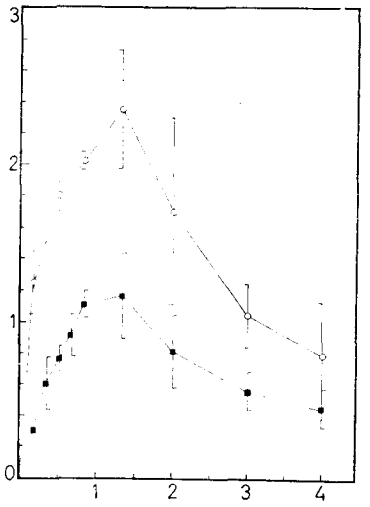

FIG. 7. Blood concentrations (ordinate: $\mu g\ ml^{-1}$) of 4-methoxybenzoic acid following administration of a dose of 1·0 mg dissolved in a lipophilic vehicle. The limits represent ± the standard error of the mean ($P = 0{\cdot}95$). ■: n-octanol. ○: isopropyl myristate. Abscissa: time (h).

hydroxybenzoic acid to rats, are shown in Fig. 7. Again, the results for isopropyl myristate are higher than those from octanol. Similar results were obtained with 4-methoxybenzoic acid. There was no simple relationship between maximum blood concentration and either k_{BC} or combinations of k_{BC} and aqueous phase concentrations in gut, but the anticipated rank order of blood concentrations was observed.

Comparison between in vitro and in vivo results thus suggests that our model can give a guide to the behaviour of a drug which is released from a water-immiscible solution in the gut. The procedure provides a simple and accurate method of comparing solute/solvent systems, and the indications are that the in vitro results are quantitatively related to the behaviour in vivo. The most interesting suggestion is that the non-aqueous solution is not in equilibrium with the gut contents, the release is dynamic, and dependent upon the rate of transfer from the one solvent to the other.

REFERENCES

Armstrong, N. A., James, K. C., Wong, C. K. (1979) J. Pharm. Pharmacol. 31

Doluisio, J. T., Crouthamel, W. G., Tan, G. H., Swintosky, J. V., Dittert, L. W. (1970) J. Pharm. Sci. 59: 72–76

Hall, T. C., Cocking, E. C. (1965) Biochem. J. 96: 626–633

McArthur, C. D. (1972) 'A user's guide to CSMP', University of Edinburgh

Muranishi, S., Kimura, T., Ogata, H., Yana, A., Inui, K., Sezaki, H., Kakemi, K. (1971) in: Kakemi, K. (ed.), 'Absorption, Metabolism and Excretion of Drugs', Hirokawa Publishing Co., Tokyo, p. 11

Rescigno, A., Segre, G. (1966) 'Drug and Tracer Kinetics', Blaisdale, Waltham, Mass. p 26

Schanker, L. S., Shore, P. A., Brodie, B. B., Hogben, C. A. M. (1957) J. Pharmacol. Exp. Ther. 120: 528–539

Stricker, H. (1971) Drugs Made Ger. 14: 93–97

Wagner, J. G. (1975) 'Fundamentals of Clinical Pharmacokinetics', Drug Intelligence Publications, Hamilton, p 58