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An *in vitro* model for soluble drug absorption

JOHN PERRIN*

A physico-chemical model is proposed to simulate the transfer of a drug through a lipoidal membrane. The drug is transferred from a buffer of a pH found in the gut, through an immiscible organic liquid acting as the membrane, to a buffer of the plasma pH (7·4). The transfer of salicylic acid and amidopyrine obeys the theoretical equations. The apparatus used has the advantage that there is little or no danger of emulsion formation because of the lack of disturbance at the interface and the ease of removing samples for analysis.

ABSORPTION of weakly acidic and weakly basic drugs is usually considered to be by passive diffusion of the unionized molecular species through the lipoidal membrane of the alimentary tract (Brodie, 1964). Correlation of physico-chemical data with *in vivo* absorption data has usually been limited to pK_a determinations, solubility measurements and measurement of distribution coefficients of the drug between an aqueous phase of physiological pH and an immiscible organic solvent. When considering a water-soluble drug it is probable that the rate of partitioning is of more interest than the distribution coefficient for comparison with *in vivo* absorption rates. This paper suggests the use of a partitioned cell based on a cell previously used for transport studies (Schulman & Rosano, 1960) for these kinetic comparisons. In Fig. 1 the drug, in solution at various pH values of the alimentary tract, is placed in compartment A and is transferred through the organic layer (simulating the lipoidal membrane) to a pH 7·4 buffer (blood pH) in compartment C. All phases are stirred to eliminate concentration gradients within the three compartments, leaving the transfer across the two interfaces the rate controlling steps. Considering the transfer to be a first order process and the concentration in the three compartments to be A, B, and C and the corresponding volumes V_A, V_B and V_C

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C$$

where k_1 and k_2 are the first order rate constants for the forward transfer and k_{-1} and k_{-2} are the corresponding back transfer constants. The transfer rates are given by

$$V_A \frac{dA}{dt} = V_A k_1 A + V_B k_{-1} B$$

$$V_B \frac{dB}{dt} = V_A k_1 A - V_B k_{-1} B - V_B k_2 B + V_C k_{-2} C$$

$$V_C \frac{dC}{dt} = V_B k_2 B - V_C k_{-2} C.$$

From the Pharmaceutical Department, I.C.I. Pharmaceuticals Limited, Macclesfield, Cheshire, England.

* Present address: School of Pharmacy, University of Wisconsin, Madison, Wisconsin 53706, U.S.A.

The solution of these differential equations is complex.* The equations are general and all transfers investigated in the cell should obey them. It is not suggested that the transfer constants obtained can be directly compared to absorption rates from animal work, but simple plots of concentration against time of various salts of a drug at different pH values, or various analogues of a drug under varying conditions, may yield data for the correlation of *in vivo* or *in vitro* studies with the gut. The purpose of the present work was to design an apparatus for measuring partitioning rates and to compare the results obtained with theoretical equations.

The rate controlling steps are the transfers across the two interfaces, which depend upon the pK_a of the drug, the interfacial area, the relative volumes of the phases, the pH values of the aqueous phases, the nature of the organic solvent and the distribution coefficient of the drug between water and the organic phase. The distribution coefficient C_o/C_w is only a true constant at a given temperature when considering the same species in both phases (Martin, 1960). Using this situation and considering single unionized drug molecules the ratio of concentrations of the drug in the two aqueous compartments at equilibrium is given by:

$$\frac{A}{C} = \frac{1 + 10^{(pK_a - pH_A)}}{1 + 10^{(pK_a - 7{\cdot}4)}} \text{ for a basic drug}$$

$$\frac{A}{C} = \frac{1 + 10^{(pH_A - pK_a)}}{1 + 10^{(7{\cdot}4 - pK_a)}} \text{ for an acidic drug}$$

and so is independent of the organic phase.

Experimental

APPARATUS

The apparatus (Fig. 1) consists of a box made of 6 mm Perspex, of internal dimensions 20 cm by 10 cm by 10 cm. The 8 cm high central partition divides the cell into two compartments of equal volume. The removable box is held rigidly on a three-screw Perspex levelling table by means of two corner brackets. The two aqueous compartments (A and C) are stirred by means of air-driven magnetic stirrers, and the top organic layer by means of a glass stirrer. Butyl stoppers are placed in 13 mm holes drilled in the middle of the end walls (35 mm from the base of the cell).

REAGENTS AND BUFFERS

All buffers were 0·2 molar: pH 2·0 buffer used Analar potassium chloride, pH 3·0 and pH 4·0 used Analar citric acid, and buffers of pH 5·0 to pH 8·0 used Analar monosodium dihydrogen phosphate—the final adjustment to pH being with concentrated hydrochloric acid or a concentrated solution of sodium hydroxide pH measured with a Model 23A pH meter Electronic Instruments Limited. The aminopyrine was B.D.H.

* For solution see: Frost & Pearson (1961), *Kinetics and Mechanism*, 2nd Edn, John Wiley.

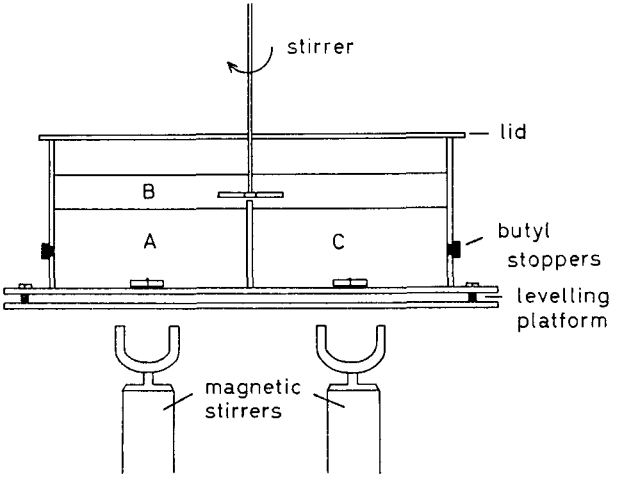

FIG. 1. Diagram of the cell.

laboratory reagent grade, the salicylic acid was Analar grade and propranolol (Inderal; 1-isopropylamino-3-(1-naphthyloxy)-2-propanol hydrochloride) was clinical trial material of I.C.I. compound 45,520. The decanol was B.D.H. laboratory reagent grade, the amyl alcohol was Analar grade and the cyclohexane B.D.H. laboratory reagent, suitable for spectroscopy.

METHOD

The cleaned cell was placed on the levelling table between the brackets and levelled by means of the three screws. Equimolar buffer solutions were prepared to prevent the cell acting as an osmometer, and the buffers were saturated with top phase to minimize transfer by one phase dissolving in the other. Approximately 20 mg of the drug was added to 1 litre of the solution to be placed in compartment A. 550 ml of this solution placed in the compartment took the level of the solution almost to the top of the central partition. A similar volume of pH 7·4 buffer was placed in compartment C. 300 ml of the top organic phase (presaturated with pH 7·4 buffer) was lowered gently onto the aqueous solutions so producing the organic "membrane" connecting the two aqueous phases. The stirring was then commenced at such a rate as to prevent vortices from forming. Samples (1·8 ml) were removed with needles and syringes via the butyl stoppers from the aqueous compartments at various time intervals. At the same time, 2 ml samples were taken from the top. Fresh top solution (2 ml) was added after removal of the top sample to prevent the level of the liquid falling below the paddles of the top stirrer. All samples were assayed by means of their ultraviolet absorption spectra using 1 cm or 4 cm microcells in the Hilger and Watts "Uvispek" spectrophotometer. The experiments were continued until equilibrium was attained or approached.

Results

EFFECT OF TOP PHASE

Fig. 2 shows the effect of the top phase on the transfer rates of propranolol (pK_a = 9·53 in ionic strength of 0·2). The free base is much more soluble in amyl alcohol than cyclohexane and so the transfer rate from the pH 8·0 phase to the organic layer is greater with the higher

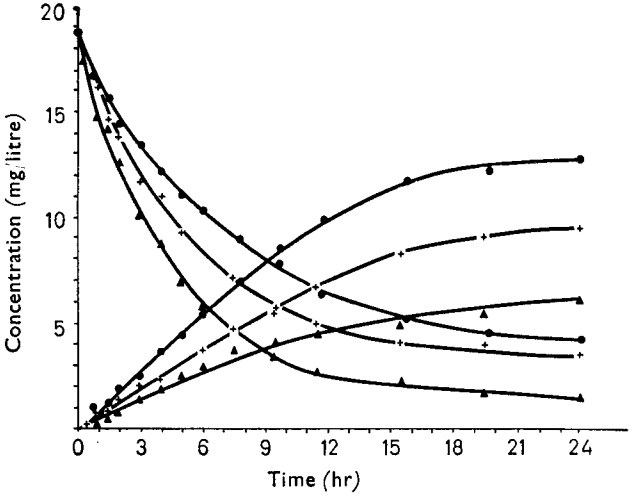

FIG. 2. Effect of the composition of the organic layer on the transfer rate of propranolol from a pH 8·0 aqueous layer to a pH 7·4 layer. ▲, 10% amyl alcohol in cyclohexane. +, 5% amyl alcohol in cyclohexane. ●, cyclohexane.

alcohol content, but the retention by this top phase is also greater and so the appearance in compartment C is slower as shown in the graph. In all instances the equilibrium ratio (3·9) of concentration in compartment C to that in compartment A was approached.

ACIDIC DRUG

Salicylic acid (pK_a 2·96 at an ionic strength of 0·21) was used and preliminary experiments suggested that 20% decanol in cyclohexane was a suitable organic phase. The distribution coefficient (63·3) was heavily in favour of the top layer. Transfer from buffers of pH 2, pH 3, pH 4, and pH 5 to a buffer of pH 7·4 was investigated. At pH 2·0 salicylic acid is present mainly as the unionized drug and as such is only sparingly soluble in the water and so quickly passes into the organic layer. However, when the drug passes to compartment C it ionizes completely and is quickly removed from B (Fig. 3), and little is retained by the top phase at equilibrium. At pH 3·0 approximately 48% of the drug is in the free acid form and this is sufficient to maintain the transfer rates. At pH values of 4 and 5 however, there is insufficient free acid to maintain the transfer rate although the drug appears in compartment C at the same

rate as at the lower pHs. It was found, with this system, that the back transfer constants are negligible and so the rate equations reduce to

$$V_A \frac{dA}{dt} = - V_A k_1 A$$

$$V_B \frac{dB}{dt} = V_A k_1 A - V_B k_2 B$$

$$V_C \frac{dC}{dt} = V_B k_2 B$$

These readily integrate to give

$$A = A_0 e^{-k_1 t}$$

$$B = \frac{550\, A_0 k_1}{300 k_2 - \frac{550}{300} k_1} \left(e^{-\frac{550}{300} k_1 t} - e^{-k_2 t}\right)$$

$$C = A_0 \left[1 - \frac{1}{k_2 - \frac{550}{300} k_1} \left(k_2 e^{-k_1 t} - k_1 e^{-k_2 t} - \frac{550}{300} k_1 e^{-k_1 t} + k_1 e^{-\frac{550}{300} k_1 t}\right) \right]$$

where $V_C = V_A = 550$ ml and $V_B = 300$ ml. Fig. 3 shows the experimental points together with the theoretical curves obtained using the

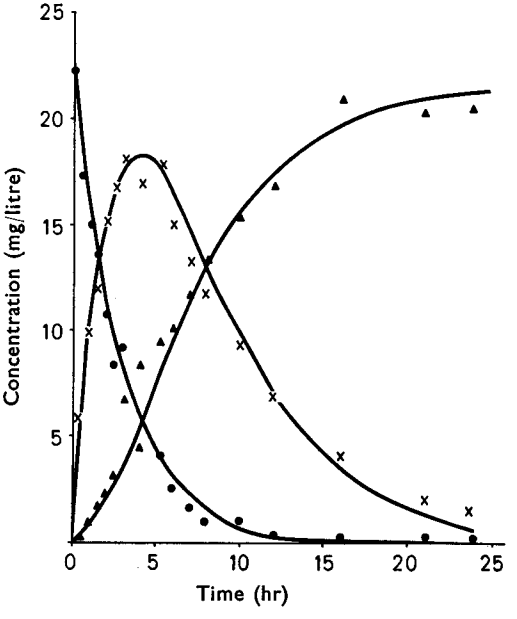

FIG. 3. Transfer of salicylic acid from pH 2·0 to pH 7·4 through a layer of 30% decanol in cyclohexane. The points are experimental and the lines are theoretical. ●, drug in pH 2·0 compartment. ×, drug in organic layer. ▲, drug in pH 7·4 compartment.

above equations in a digital computer. The computer derived rate constants are:

pH	k_1 (hr^{-1})	k_2 (hr^{-1})
2	0·34	0·22
3	0·37	0·22
4	0·12	0·22
5	0·044	0·25

BASIC DRUG

Amidopyrine (pK_a 5·10 at ionic strength 0·21) was taken as the example and cyclohexane was used as the top phase. The partition coefficient (0·213) was in favour of the water. Here the transfer rate from A to B rises as expected but the overall picture is complicated by the back transfer rate constants which are not longer negligible. This is due to the fact that the drug is more ionized in A than C and to the value of the distribution coefficient. The drug in all experiments built up quickly to a low equilibrium value in the organic layer, but the overall equilibrium was approached very slowly. Fig. 4 shows a typical set of results together

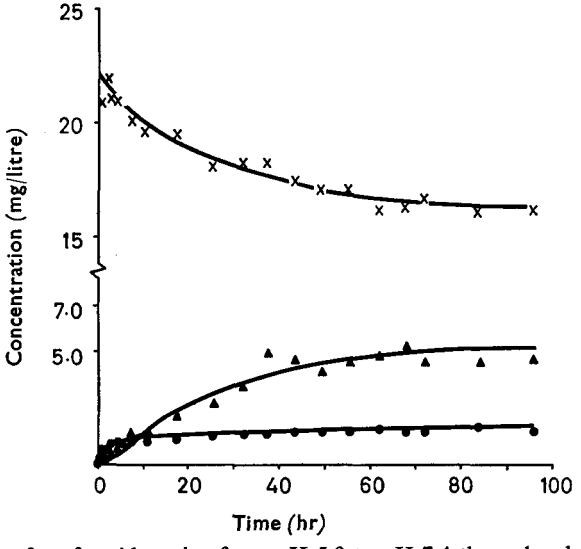

FIG. 4. Transfer of amidopyrine from pH 5·0 to pH 7·4 through a layer of cyclohexane. The points are experimental and the lines are theoretical. ×, drug in pH 5·0 compartment. ●, drug in organic layer. ▲, drug in pH 7·4 compartment.

with the theoretical curves derived using the general equations. The estimates of the transfer rates (hr^{-1}) obtained by the digital computer are:

pH	k_1	k_{-1}	k_2	k_{-2}
5	0·018	0·33	0·34	0·055
6	0·045	0·35	0·46	0·082
7	0·058	0·39	0·44	0·075

Discussion

The results show the design and construction of the apparatus to be adequate in that the data obey the theoretical equations. If the system is to be compared to drug transfer across a lipoidal membrane there should be little or no retention by the organic layer. The amount retained will be controlled by the pK_a of the drug, the pH values of the aqueous phase and the distribution coefficients. These problems are illustrated by the examples cited above and are usually more difficult to overcome with basic drugs particularly when using physiological pH in compartment A. Frequently solvents with no similarity to natural lipids, such as chloroform and cyclohexane, are used in partition studies for correlation with drug absorption data. Higher alcohols such as decanol and dodecanol can be used in this apparatus without the danger of emulsion formation, which can occur in simple partitioning and in the three phase rocking apparatus (Doluisio & Swintosky, 1964). Again this lack of emulsion formation enables natural lipids such as lecithin and cephalin to be dissolved in the top phase, and these materials are known to orientate at an oil-water interface with the polar head towards the aqueous phase. In this situation the rate controlling steps would be the transfer across this lipid interfacial "membrane," so giving a much closer comparison to the natural process. Work is continuing in this direction.

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