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An *in vitro* model for drug absorption studies

J. S. ROBERTSON AND O. BODE

Department of Pharmacy, University of Sydney, Sydney, N.S.W. 2006, *Australia*

The transfer of salicylic acid and of three sulphonamides from an aqueous phase of pH 2 or pH 5 through an intervening organic phase to an aqueous phase of pH 7·4 has been studied using a rotating cell. The manner of operation of the cell promotes rapid drug transfer without vortexing or emulsification of the phases. The rates of transfer of the drugs showed the anticipated pH dependance.

The gastrointestinal absorption of drugs is often dependent upon their ability to penetrate a lipid barrier; for some compounds absorption is accomplished by passive diffusion of the unionized moiety (Brodie, 1964).

In vitro experimental techniques intended to mimic *in vivo* absorption behaviour have been described by Doluisio & Swintowsky (1964), and Perrin (1967). In general, in the apparatus used in these techniques the compound being examined is in solution in one of two aqueous phases that are buffered to suitable pH values, e.g. pH 2 or pH 5 to simulate stomach pH, and pH 7·4 to simulate the pH of the plasma. The aqueous phases are separate from each other and are overlaid by an organic phase (simulating a lipid barrier) which is in contact with both aqueous phases.

In the Perrin apparatus, the transfer rate is limited by the fixed interfacial area and by the tendency of the phases to mix by vortexing when stirred too rapidly. In the Doluisio–Swintowsky apparatus the interface alternately expands and contracts in a rather narrow tube; too rapid rocking tends to promote emulsification. The apparatus described here is of the same general design but has continuously generated interfaces allowing rapid interchange of drug between the aqueous and organic phases without vortexing or emulsification of the phases. The kinetics of transfer of salicylic acid and of some sulphonamides have been studied in the apparatus and are reported.

EXPERIMENTAL

Apparatus

The apparatus (Fig. 1a and b) consists of a circular, partitioned cell which is slowly rotated on rollers. The cell body is made of two pieces of copper tubing 6·3 cm long, 12·7 cm in diameter and 3·2 mm wall thickness, threaded at each end, and coated internally with "Vitrenite",* to protect the walls against chemical attack. The half cells are joined together by a partition made from a block of Perspex that has been suitably shaped and threaded on both faces, and having a central hole of 5·6 cm diameter. The end pieces are of Perspex, suitably threaded, with a hole of 1·4 cm diameter drilled 1 cm in from the edge; these holes are plugged with the type of rubber stopper used to seal multidose vials. A small hole drilled through the centre of one of the end pieces allows air to enter the cell, when sampling. The seams between Perspex and metal are sealed externally with a water-resistant glue. The cell is rotated at 32 rev/min on rubber-covered rollers driven by a 110 W constant

* Metal Protectives Co. Pty. Ltd., 1 Hamilton Street, Granville, N.S.W. 2142.

speed motor. (A variable speed motor would give greater flexibility of operating conditions.) The rollers are long enough to accommodate six cells at once.

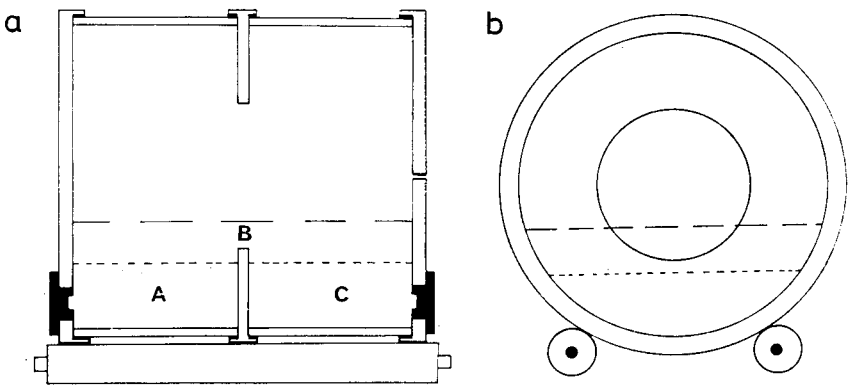

FIG. 1. a. Sectional drawing along length of circular cell. b. Sectional drawing along diameter of cell.

Materials

Salicylic acid, sulphacetamide sodium, sulphadiazine sodium and sulphadimidine sodium were of B.P. standard. Cyclohexane was supplied by Ajax Chemicals Ltd., Sydney, and octan-2-ol by British Drug Houses, U.K. The buffer solutions of pH values 2, 5 and 7·4 were those of the British Pharmacopoeia, p. 1209. Buffer solutions were equilibrated with the organic phase before use.

Salicylic acid was determined spectrophotometrically. The sulphonamides were determined by the Bratton & Marshall technique (Bratton & Marshall, 1939).

Method

100 ml of buffer solution pH 2 or pH 5 containing the drug was placed in compartment A (Fig. 1a), and 100 ml of buffer solution pH 7·4 was placed in compartment C. The aqueous phases were carefully overlaid with 200 ml of organic phase (compartment B). The cell was rotated on the rollers, and samples were removed by syringe via the plugs at suitable time intervals and assayed.

RESULTS

As can be seen from Fig. 2A, salicylic acid (pK_a 2·97) was readily transferred from an aqueous phase of pH 2, through a cyclohexane layer to an aqueous phase of pH 7·4, where it was almost entirely ionized and trapped. Since all of the salicylic acid in the system was found entirely in the aqueous phases, the transfer could be described symbolically as $D_A \rightarrow D_C$ (Doluisio & Swintowsky, 1965), where D_A is the drug concentration in the pH 2 phase, and D_C the concentration in the pH 7·4 phase. A first order plot of the data from Fig. 2A yielded a straight line.

The dependance of amount of drug transferred on pH of the aqueous phase is shown in Fig. 2B; the concentration of unionized drug at a pH of 5 is approximately 1%.

The influence of the polarity of the organic phase is also shown in Fig. 2B; addition of 1% v/v octan-2-ol to the cyclohexane markedly increased the rate of transfer.

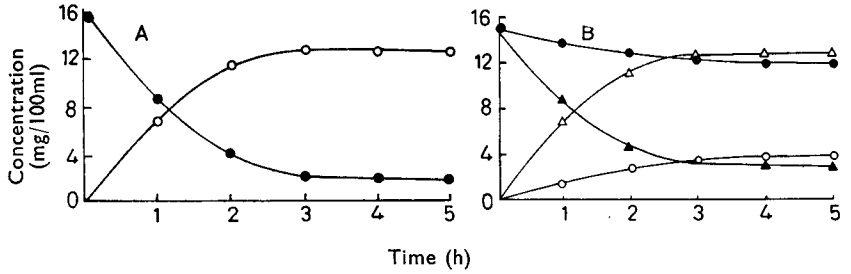

FIG. 2. A. Transfer of salicylic acid from pH 2 phase through a barrier of cyclohexane to a pH 7·4 phase. ● pH 2 phase. ○ pH 7·4 phase.

B. Effect of pH and of polarity of the organic phase on the transfer rate of salicylic acid from pH 5 phase to pH 7·4 phase. ● Compound in pH 5 phase (cyclohexane only). ○ Compound in corresponding pH 7·4 phase. ▲ Compound in pH 5 (cyclohexane plus 1% v/v octan-2-ol). △ Compound in corresponding pH 7·4 phase.

The transfer characteristics of sulphacetamide, sulphadiazine and sulphadimidine were examined; these drugs each have two ionizable groups, the pK_a values of which are: sulphacetamide,* 1·4, 5·38; sulphadimidine,* 2·76, 7·37; sulphadiazine,† 2·24, 6·42.

The percentages of unionized species present between pH 2 and pH 7·4 were calculated from the formula:

$$\% \text{ unionized} = \frac{1}{1 + 10^{pK-pH} + 10^{pH-pK}} \times 100.$$

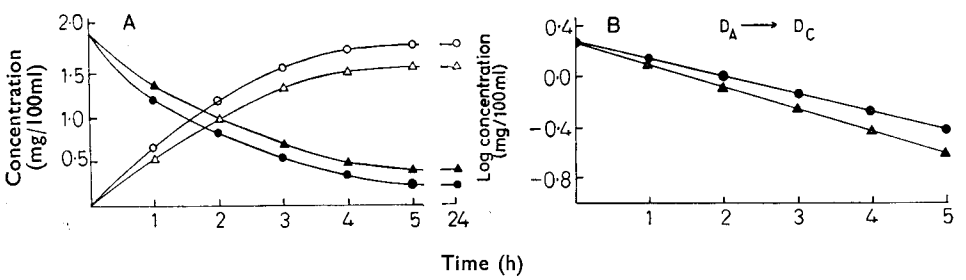

FIG. 3. A. Transfer of sulphacetamide from pH 2 phase and pH 5 phase through a barrier of cyclohexane-octan-2-ol (35:65 v/v) to a pH 7·4 phase. ● pH 2 phase. ○ Corresponding pH 7·4 phase. ▲ pH 5 phase. △ Corresponding pH 7·4 phase.

B. Plot of data in Fig. 3A, showing apparent first order disappearance of sulphacetamide from phases pH 2 and pH 5. ● pH 2 phase. ○ pH 5 phase.

The results of the transfer studies (Figs 3 and 4) showed that sulphacetamide behaved in a manner similar to that of salicylic acid, i.e. in a given time more compound was transferred from a pH 2 phase than from a pH 5 phase. However the difference between the amounts so transferred was much less than with salicylic acid. A first order plot of the data from Fig. 3A yielded straight lines (Fig. 3B).

With both sulphadiazine and sulphadimidine Fig. 4A,B there was a greater amount of drug transported at pH 5 than at pH 2. This behaviour reflects the concentration of unionized species of each drug available for transfer at the chosen pH values.

* Data taken from "The Sulphonamides and Allied Compounds".

† Data supplied by Dr. Brian Rawson of this Department.

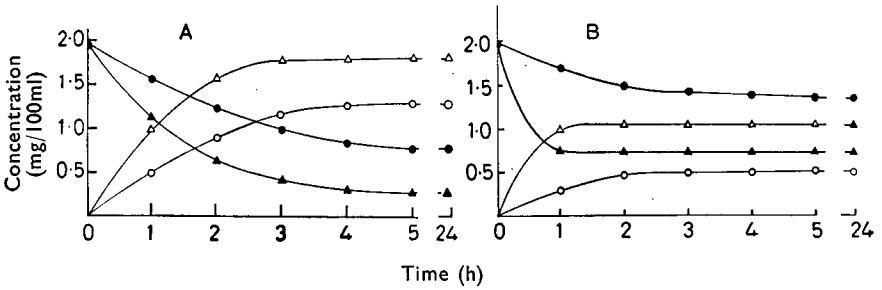

FIG. 4. Transfer of (A) sulphadiazine and (B) sulphadimidine from pH 2 phase and pH 5 phase through a barrier of cyclohexane-octan-2-ol (35:65 v/v), to a pH 7·4 phase. ● pH 2 phase. ○ Corresponding pH 7·4 phase. ▲ pH 5 phase. △ Corresponding pH 7·4 phase.

Moreover, first order plots of the transfer data for these two drugs did not yield straight lines, but curves. However, since all of each drug was contained only in the aqueous phases, a plot of $D_A - D_A^{\infty}$ against time should yield straight lines (Doluisio & Swintowsky, 1965). D_A = concentration of drug in compartment A at any time, and D_A^{∞} = equilibrium concentration of drug in compartment A). Straight line graphs were obtained (Fig. 5).

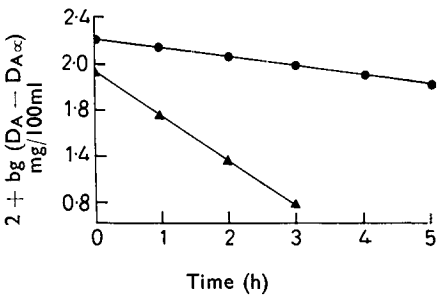

FIG. 5. Plot D_A–D_A^{∞} for data in Fig. 4, pH 5 phase only. ● Sulphadiazine. ▲ Sulphadimidine.

These results indicated that the apparatus described was satisfactory for studying the transport of drug molecules between aqueous phases via a lipid phase.

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