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A note on the diffusion of drugs through artificial phospholipid membranes

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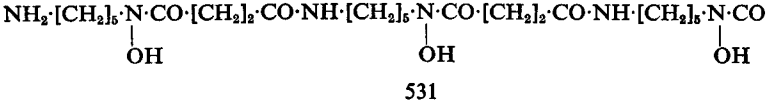
A relatively simple method for the assessment of the passive diffusion of drugs through artificial phospholipid membranes composed of lecithin and collodion yields data on the permeability of some pharmaceutical substances through lipid barriers. Although there are inherent limitations in using artificial phospholipid membranes as substitutes for more complex biological membranes, this model system can furnish information on permeability kinetics and on the effect of structural groups on biological activity.

MANY efforts have been made in the last three decades to devise simpler models of biological membranes for the examination of transport phenomena. Membrane permeability and equilibria have been discussed by Meyer & Sievers (1936), Adair (1937), Meyer (1937), Teorell (1937) and Wilbrandt (1959); the kinetics of diffusion (Laidler & Shuler, 1949; Zwolinski, Eyring & Reese, 1949); penetration (Davson & Danielli, 1952) and thermodynamics of irreversible processes (Kedem & Katchalsky, 1958; Katchalsky, 1960) have been reported. After we had completed our work the review of Lakshminarayanaiah (1965) on transport phenomena in artificial membranes appeared. But there have been comparatively few direct studies on the permeability of drugs through artificial phospholipid membranes. A qualitative study on the permeability of collodion-beef brain lipid membranes by Weatherby (1943, 1949) showed that these membranes displayed asymmetry potentials which varied according to the pH of the fluid to which they were exposed and that they possessed a high degree of permeability to the lipid-soluble undissociated molecules of nicotine and salicyclic acid. Changes in degree of ionisation by structural modifications also changed the rate of permeation through these membranes. Brodie (1964), and Schanker, Tocco, Brodie & Hogben (1961), observed that gastrointestinal epithelium, renal tubular epithelium, blood brain barrier, and boundaries of various tissue cells, acted as lipid-like barriers preferentially permeable to lipid-soluble undissociated drug molecules with rates of transfer related to lipid-water partition coefficients and dissociation constants of drugs. It therefore appeared of interest to obtain data on the permeability characteristics through collodion-lecithin membranes of a series of biologically interesting compounds available in our laboratories. These were: ferrioxamine-B hydrochloride* (Bickel, Hall, Keller-Schierlein, Prelog, Vischer & Wettstein, 1960), glutethimide (Tagmann, Sury & Hoffman, 1952), thalidomide, salicylic acid, morphine, nalorphine and

From CIBA Ltd., Basle, Switzerland.

* Ferrioxamine-B is the Fe^{3+} complex of deferrioxamine, an amphoteric compound with one strongly basic aliphatic amino-group and three weakly acidic hydroxamic acid groups. The latter has the structure:



2,4-di(diethylamino)-6-(2-phenylacetylhydrazino)-1,3,5-triazine, a potent blocker of polysynaptic reflexes and a depressant of muscle spindle activity (Bein & Fehr, 1962).

Experimental

Purification of lecithin. The lecithin was purified egg lecithin (Merck) which was freed of cephalin by passing it in a methanolic solution through a column of Dowex 1 × 4 (50–100 mesh) resin in bicarbonate form according to the method of Perrin & Saunders (1960). The lecithin passed through and the cephalin was held on the resin column. The product was further purified using an ethyl methyl ketone-acetone mixture and lecithin was obtained as a waxy mass having an N:P ratio of 1·01. Weatherby (1943, 1949) used an ill-defined mixture of soybean and brain phosphatides.

Preparation of collodion-lecithin membranes. The method of preparation of membranes was similar to that used by Weatherby (1943). A 10 ml aliquot of a solution of collodion (1%) and lecithin (1%) in absolute ether-absolute ethanol (9:1) was pipetted over clean mercury contained in a petri dish of 110 mm diameter, the solvent was allowed to evaporate under controlled conditions for about 9 hr. The membrane was then cut out and immersed overnight in distilled water until ready for use. All membranes were prepared under similar controlled conditions. Their average thickness was 20 ± 2 μ using a Mikrotest magnetic thickness measuring instrument.

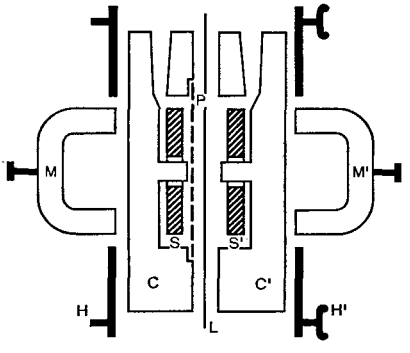


FIG. 1. Diffusion cell assembly. CC′, circular glass cells with ground edges. SS′, stirrers rotating on vertical axes. MM′, magnets (synchronised). L, collodion-lecithin membrane. P, supporting round metal plate with circular holes. HH′, aluminium circular holders with tightening screws.

Method of measuring diffusion characteristics. The diffusion experiments were run in the apparatus shown in Fig. 1 (the design of apparatus kindly supplied by Dr. Majer of CIBA Ltd., Basle). It consists of two circular ground glass cells CC′ of capacity approximately 20 ml each. The cells are provided with magnetic stirrers SS′ for continuous stirring of solutions in the compartments by synchronised motor. The membrane

is supported in place by a thin stainless steel round plate P with circular holes. After setting the membrane in place, cells are screwed together by two round aluminium gaskets on either side and mounted on a support assembly. A known concentration of drug, in buffers of known pH, was used on the right side and an equal volume of same buffer on the left side. In some experiments, an equal volume of distilled water was used in the left compartment. After suitable intervals, an aliquot of fluid was analysed. The diffusion of most compounds was studied in 0·1 m phosphate buffer pH 7·4, that of salicylic acid at two other pH values 2·5 and 8·5.

Estimations. Phenol red and salicylic acid were estimated by the method of Schanker & others (1958), aniline by Bratton & Marshall's method (1939), ferrioxamine-B by Tripod & Keberle's method (1962), glutethimide-^{14}C and thalidomide-^{14}C by liquid scintillation counting, Val^5-angiotensin-Asp-β-amide by paper chromatography, morphine and nalorphine by ultraviolet spectrophotometry in N hydrochloric acid solutions of appropriate dilutions.

Results and discussion

The compounds that did not diffuse through a collodion-lecithin membrane from a solution in 0·1M phosphate buffer at pH 7·4 buffer to water were (conc. μg/ml): phenol red (100), ferrioxamine-B hydrochloride (1600), *N*-[2-(β-ethoxyphenyl)acetyl]ferrioxamine-B hydrochloride (1600) and Val^5-angiotensin-Asp-β-amide (1000). This last compound showed marked degradation to peptides and amino-acids. These compounds also do not pass through *in vitro* rat intestinal preparations in significant concentrations (Misra, Hunger & Keberle, 1966).

The diffusion of all substances in Table 1, except thalidomide which has limited solubility in phosphate buffer pH 7·4, was studied at 1 mmolar concentration. The permeability constants for these compounds were obtained by using the equation derived by Lueck, Wurster, Higuchi, Lemberger & Busse (1957) for describing a diffusion process under quasi-steady state conditions in which two well-stirred liquids, either or both containing a solute, are separated by a barrier permeable to the solute:

$$\log (C_o - 2\,C_b) = -\frac{2\,K}{2{\cdot}303}\,t + \log C_o$$

where C_0 is the initial concentration of the penetrant in the solution on the side of origin, C_b the concentration of penetrant in receiving chamber, t the time of sampling. K is the permeability constant of the membrane, which, according to Lueck & others (1957), is defined as $K = A.D.(DC)/V.L$, where A is the cross-sectional area of the membrane; L is the thickness of the membrane; V is the volume of each of the two chambers; D is the diffusion coefficient; (DC) is the distribution coefficient between solution and membrane.

A plot of log ($C_o - 2\,C_b$) against time of sampling gave a straight line with a slope equal to $-2K/2{\cdot}303$ for at least 4 hr with the exception of salicyclic acid in acid medium.

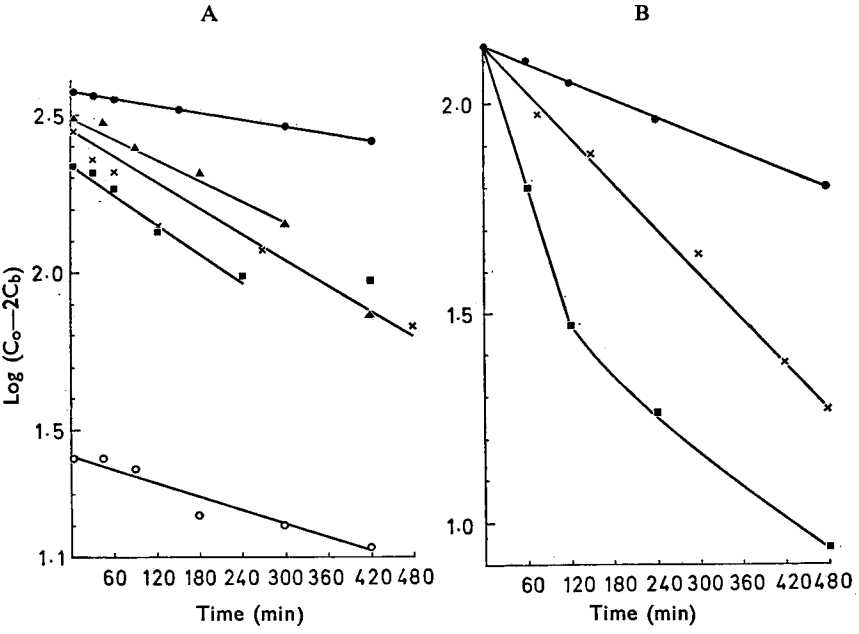


FIG. 2A. Diffusion drugs from solutions through a collodion-lecithin membrane. ●——● 2,4-Di(diethylamino)-6-(2-phenylacetylhydrazino)1,3,5-triazine 1 mmolar solution in 0·01 N hydrochloric acid; ▲——▲ nalorphine; ×——× morphine hydrochloride; ■——■ glutethimide-^{14}C; ○——○ thalidomide-^{14}C all in 0·1 mmolar solution in 0·1 M phosphate buffer pH 7·4.

B. Diffusion of salicylic acid from 1 mmolar solutions of different pH values through collodion-lecithin membrane. ●——● 0·1 M Borate buffer pH 8·5; ×——× 0·1 M phosphate buffer pH 7·4; ■——■ 0·1 M citrate buffer pH 2·5.

These plots are given in Fig. 2A. With salicylic acid (Fig. 2B) at pH 2·5 a straight line relationship is obtained only for the first 2 hr probably due to adsorption of acid on membrane.

Salicylic acid diffused two to three times faster at pH 7·4 and about eight times faster at pH 2·5 than at pH 8·5 (Table 1). Nalorphine diffused through collodion-lecithin membrane at pH 7·4 at a rate comparable to morphine. The triazine derivative diffused approximately 15 times slower at pH 2·0 than did salicylic acid at pH 2·5. The limited solubility at physiological pH of the triazine derivative provided indirect though insufficient evidence that the stomach could be an important site for its absorption. Glutethimide-^{14}C diffused more slowly than salicyclic acid at pH 7·4 and twice as fast as thalidomide-^{14}C. Glutethimide-^{14}C also permeated faster through *in vitro* rat intestinal preparations than did thalidomide-^{14}C.

The limited solubility of many pharmacologically potent lipid-soluble drugs in buffers and the affinity with which some drugs are adsorbed and held on the phospholipid membrane impose some limitations on the present method.

TABLE 1. PERMEABILITY CONSTANTS OF DRUGS THROUGH COLLODION-LECITHIN MEMBRANES

Compound	Conc. used (μg/ml)	pH of buffer used	Permeability constant ($K \times 10^6$) (min^{-1})
Salicylic acid	138	8·5	82
	138	7·4	205
	138	2·5	643
2,4-Di(diethylamino)-6-(2-phenyl-acetylhydrazino)-1,3,5-triazine ..	371·5	2·0	41
Nalorphine	311	7·4	120
Morphine hydrochloride	285	7·4	159
Glutethimide-^{14}C	217	7·4	177
Thalidomide-^{14}C	25·8	7·4	84

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