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The effect of bile salts on the interfacial transport of phenothiazines

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Summary

The transport of phenothiazine drugs across isopropyl myristate (IPM) membranes has been studied using the rotating diffusion cell. Interfacial transport rate constants across aqueous–IPM and IPM–aqueous interfaces have been calculated for mequitazine and perphenazine. Although the interfacial barriers were not rate-limiting, they were significant. The rate-limiting barrier to the mequitazine and perphenazine transport was found to be the diffusion across IPM.

The effect of bile salts on the transport of mequitazine across IPM was investigated. The hypothesis that ion-pairing may increase the transport of drugs is not supported by this study.

Introduction

Stokes (1958) has shown that a stable interface could be established on the surface of a sinter in a Stokes cell. Recently, the rotating diffusion cell (RDC) has been developed to study the kinetics of interfacial transfer of solutes (Albery et al., 1976; Albery and Hadgraft, 1979b). Before the development of the RDC, an attempt was made to study interfacial transfer kinetics in two phase systems (Albery et al., 1974; Yunker and Borodkin, 1971; Schumacher and Nagwekar, 1974). In the Stokes cell used by Albery et al. (1974), the sinter is about 3 mm thick and therefore has a relatively long response time. The RDC design has two advantages over the

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traditional Stokes cell, in that it firstly utilizes thinner membranes, therefore reducing the response time and secondly enables faster interfacial transfer rates to be measured.

Further, the RDC has been employed in the modelling of percutaneous absorption (Albery and Hadgraft, 1979a; Albery and Hadgraft, 1979b), in the estimation of diffusion coefficients (Guy and Fleming, 1979a) and in the study of permeability of a phospholipid barrier (Guy and Fleming, 1979b).

The RDC uses the hydrodynamics of the rotating disc system (Riddiford, 1966), and the transport of solute to and from the membrane is controlled by the hydrodynamics of the cell. Before the development of the RDC it had been difficult to separate and determine the interfacial rate constants at the adsorption and desorption interfaces of 3 phase systems. This paper reports on the interfacial transport of phenothiazines and as ion-pairs with bile salts across a biomembrane model using the RDC.

Materials and methods

Materials

The following companies kindly donated the phenothiazine drug samples: May and Baker, Dagenham, U.K. donated promethazine hydrochloride, trimeprazine tartrate, prochlorperazine mesylate and chlorpromazine hydrochloride; Wyeth Laboratories, Maidenhead, Berks, U.K. donated promazine hydrochloride; Squibb and Sons, Merseyside, U.K. donated fluphenazine hydrochloride; Allen and Hanburys, Bethnal Green, London, U.K. donated perphenazine base; and Berk Pharmaceuticals, Guildford, Surrey, U.K. donated mequitazine base. All the phenothiazine derivatives were of pharmaceutical grade and used without further purification.

Bile salts were obtained from the following sources: sodium cholate (Na.C), Widespread, London; sodium deoxycholate (Na.D), Kochlight Labs., Bucks.; sodium taurodeoxycholate (Na.TDC) and sodium glycocholate (Na.GC), Sigma Chemicals; sodium glycodeoxycholate (Na.GDC), Calbiochem, San Diego, U.S.A.

Methylnicotinate was donated by Berk Pharmaceuticals and isopropyl myristate (IPM) was donated by Croda Food Ingredients, Cheshire. Both were used as supplied.

Methods

The RDC of Albery et al. (1976) was used to determine the interfacial transport of phenothiazines across IPM contained in Nuclepore polycarbonate membranes (Nuclepore Corporation, CA, U.S.A.). The membranes of 0.1 μm pore diameter, 5 μm thick and a porosity of 2.4% were attached to the cell with an adhesive made by dissolving perspex in butanone. The membrane was saturated with IPM and any excess carefully removed. A new membrane was used for each phenothiazine. The concentration of the phenothiazine donor solution used was 0.005% w/v. The donor and receptor volumes were 30 ml and 150 ml, respectively. With all phenothiazines,

the transport across IPM was monitored continuously by ultraviolet (UV) spectroscopy at the corresponding maximum wavelength of absorption. The transport was followed for 4 h for each phenothiazine drug and at each rotation speed.

The transport of mequitazine in the presence of bile salts was followed across a similar IPM-impregnated membrane. The concentration of mequitazine hydrochloride and that of bile salts used in this study was 1.4×10^{-4} M. At this concentration, micellization is excluded (Murthy and Zografi, 1970), and the solubility product of the ion-pair complex has not been exceeded.

The determination of diffusion coefficients (D_{org}) of mequitazine and perphenazine in IPM was carried out using the modified Stokes cell of Kreevoy and Wewerka (1967). Methylnicotinate was used as a standard solute in calculating D_{org} for mequitazine and perphenazine (Ahmed, 1981).

RDC theory and calculation of interfacial rate constants

The detailed theory of the RDC has been previously described (Albery et al., 1976). Briefly, however, the rotation of the RDC produces two stagnant diffusion layers, one on each side of the membrane and these are defined by the hydrodynamic flow pattern induced by the rotation.

The rate of transfer ($J \cdot mol \cdot s^{-1}$) of the diffusing species from the inner to the outer compartment is given in Eqn. 1.

$$J = \bar{k}AC_t \tag{1}$$

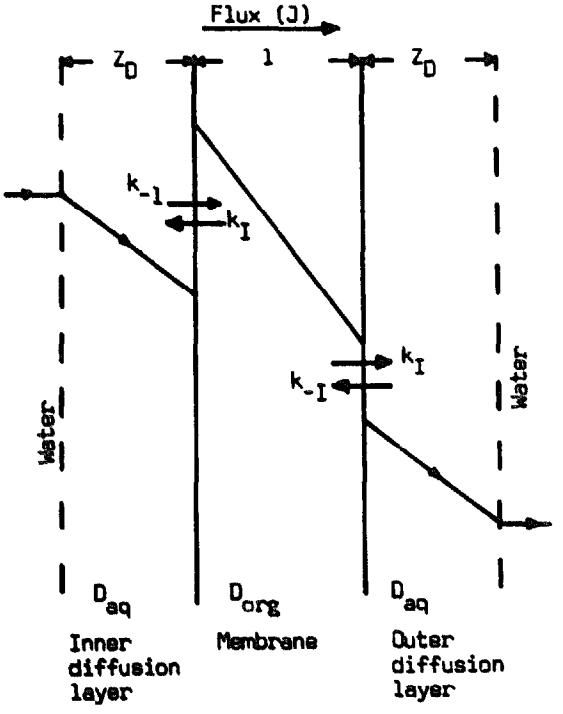

Fig. 1. Double interface system: concentration profile from inner to outer compartment.

where A is the area of membrane available for permeation, C_I is the concentration in the inner compartment and $\vec{k}$ is the forward rate constant given by

$$\frac{1}{\vec{k}} = \frac{2 \cdot Z_D}{D_{aq}} + \frac{2}{\alpha \cdot k_{-1}} + \frac{Kl}{\alpha \cdot D_{org}} \tag{2}$$

where α is the area of the pores of the membrane divided by A and $K = k_1/k_{-1}$. Other symbols are as in Fig. 1.

The term $2 \cdot Z_D/D_{aq}$ describes diffusion through the aqueous stagnant diffusion layers, $2/\alpha \cdot k_{-1}$ describes the interfacial transfer reactions and $Kl/\alpha \cdot D_{org}$ describes diffusion through the membrane of thickness, l. The aqueous stagnant diffusion layer, Z_D is given by the Levich equation (Levich, 1962).

$$Z_D = 0.643\omega^{-1/2}\nu^{1/6}D_{aq}^{2/3} \tag{3}$$

where ω (Hz) is the rotational speed and ν (m^2s^{-1}) is the kinematic viscosity. On substituting Eqn. 3 for Z_D into Eqn. 2, the expression

$$\frac{1}{\vec{k}} = \frac{1.286\omega^{-1/2}\nu^{1/6}}{D_{aq}^{2/3}} + \frac{2}{\alpha \cdot k_{-1}} + \frac{Kl}{\alpha \cdot D_{org}} \tag{4}$$

is obtained, which in turn is used to calculate interfacial transfer rate constants and D_{aq} for phenothiazine from the plots of $\vec{k}^{-1}$ versus $\omega^{-1/2}$.

Results and discussion

The assessment of the rate of transport of drugs is of importance, since the onset of action is governed by the transport of the drug to its site of action. Fig. 2 shows plots of $\vec{k}^{-1}$ against $\omega^{-1/2}$ for the 8 selected phenothiazines. The transport rate of all the phenothiazines across IPM is increased with increasing rotational speed. The gradients and intercepts were obtained from lines fitted by linear regression analysis to experimental points.

Before proceeding to investigate the interfacial transport rate constants, the effect of temperature on the transport process was investigated since temperature is one of the most fundamental of all factors that affect rate processes, although in biological systems it may be considered as constant. The mequitazine transport rate increased with increasing temperature. The activation energy of mequitazine transport across IPM was calculated to be 27.1 $kJ \cdot mol^{-1}$ from the gradient of the Arrhenius plot (Fig. 3). Further, perphenazine was selected to study the effect of pH on transport, since perphenazine has a second pK_a of 7.8. Fig. 4 shows the transport rate–pH profile for perphenazine hydrochloride across IPM. The transport is increased with increasing pH and perphenazine transport across IPM does appear to obey the pH-partition hypothesis. The increased pH has the effect of increasing the unionized

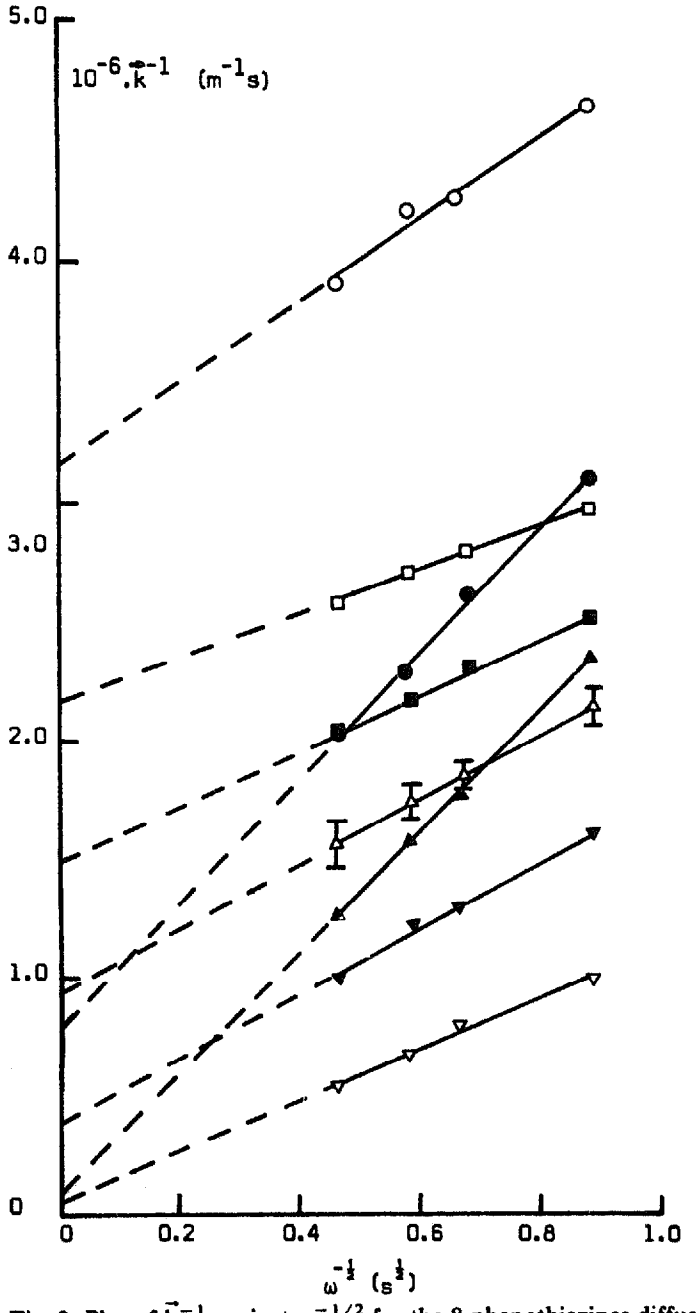

Fig. 2. Plot of $\vec{k}^{-1}$ against $\omega^{-1/2}$ for the 8 phenothiazines diffusing through IPM-impregnated nuclepore membrane. The bar on the mequitazine line indicates the standard error of mean, and is typical of the other phenothiazines. ○, chlorpromazine; ●, trimeprazine; □, promethazine; ■, prochlorperazine; ▲, promazine; △, mequitazine; ▼, fluphenazine; ▽, perphenazine.

form of drug (Fig. 4) which favours partitioning into IPM, and hence a faster transport rate.

Mequitazine and perphenazine hydrochlorides were selected from the 8 phenothiazines and their interfacial kinetics were studied in detail. The thickness of the diffusion layer, Z_D varies with $\omega^{-1/2}$ (from Eqn. 3) and $\vec{k}^{-1}$ varies with Z_D (from

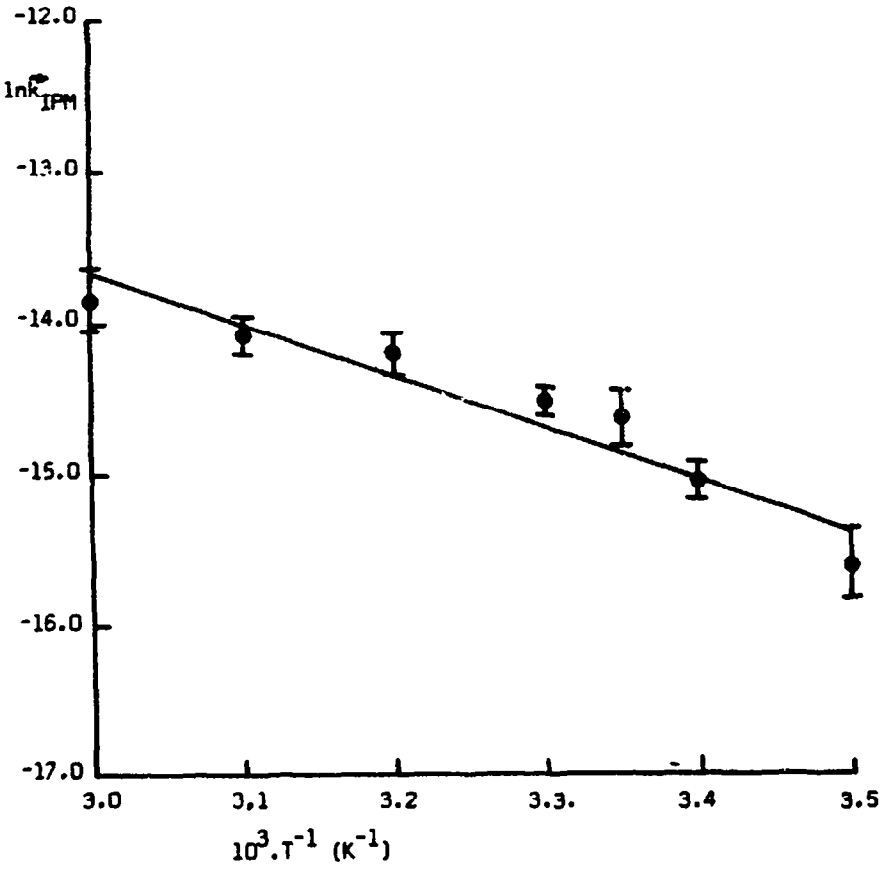

Fig. 3. Arrhenius plot for the transport of mequitazine across IPM. Line fitted by linear regression analysis with $r = 0.92$.

Eqn. 2). Therefore, a plot of $\vec{k}^{-1}$ should vary linearly with $\omega^{-1/2}$. Fig. 2 shows typical plots of $\vec{k}^{-1}$ against $\omega^{-1/2}$ for mequitazine and perphenazine together with other phenothiazines. The intercepts in Fig. 2 correspond to an infinite rotation speed, where $\omega^{-1/2} \rightarrow 0$ and the effects of the stagnant diffusion layers disappear. Using the extrapolated intercept, interfacial transport rate constants were calculated for mequitazine and perphenazine, and the values are shown in Table 1. The gradients of the lines allow the determination of aqueous diffusion coefficients of

TABLE 1

MEQUITAZINE AND PERPHENAZINE TRANSPORT PARAMETERS AS OBTAINED FROM THE RDC

Drug	D_{org} ($m^2 \cdot s^{-1}$)	D_{aq} ($m^2 \cdot s^{-1}$)	K	k_{-1} ($m \cdot Ms^{-1}$)	k_1 ($m \cdot Ms^{-1}$)
Mequitazine	1.17×10^{-11}	2.54×10^{-11}	0.014	121	1.67
Perphenazine	1.41×10^{-11}	3.85×10^{-11}	0.001	2110	2.31

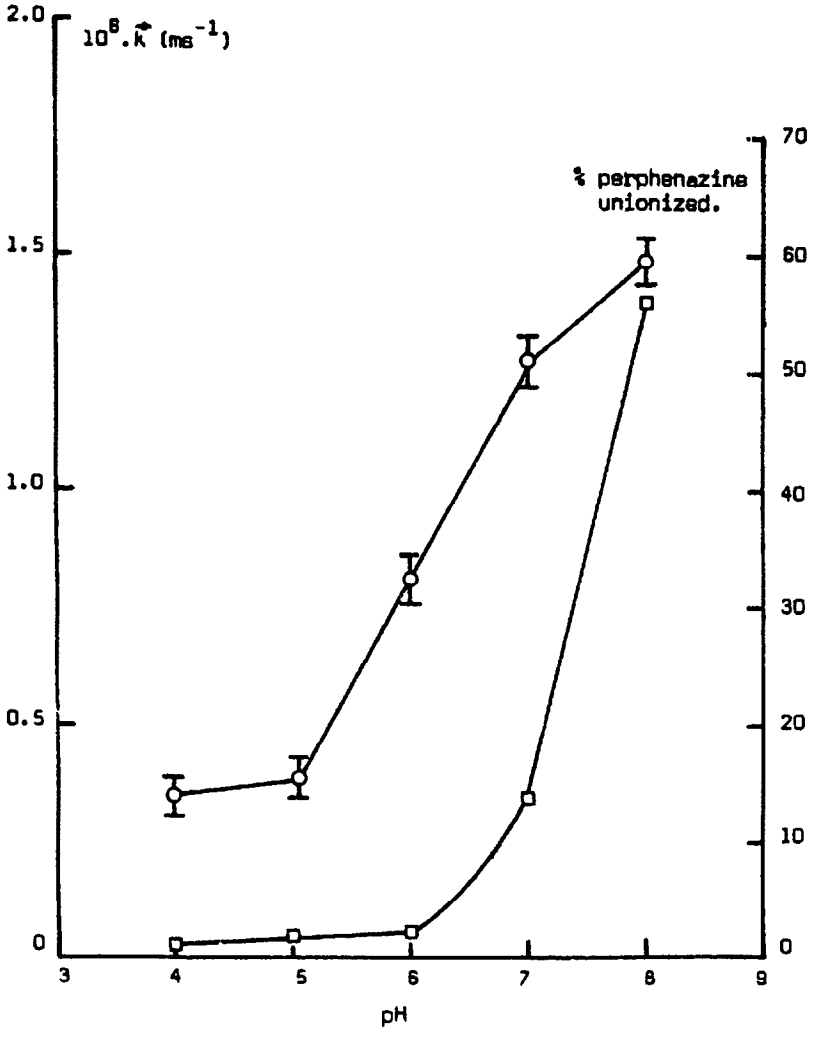

Fig. 4. The effect of pH on % perphenazine unionized and on the transport rate of perphenazine hydrochloride across IPM at 37°C. The bar indicates the standard error of mean.

solutes under investigation and the values for mequitazine and perphenazine are listed in Table 1. The partition coefficients (K) of mequitazine and perphenazine required in calculating the interfacial transport rate constants were determined from the relative solubilities in the aqueous and organic phases (Guy and Fleming, 1979a). This method of determining K was used because of the precipitation of the drug and phospholipids in the aqueous phase (Guy and Fleming, 1979a).

The separation of the overall transport rate constant across IPM into k_{-1}, k_1 and D_{org} permits illucidation of the rate controlling factors of solute transport across membranes. If the magnitude of the interfacial barrier is compared with the organic diffusion barrier in terms of reciprocal permeabilities (Flynn et al., 1974), it can be seen that

$$\frac{1}{K \cdot k_{-1}} > \frac{1}{D_{org}}$$

for interfacial barriers to become rate-limiting. The above inequality is applicable to thin membranes (< 100 μm) including biological membranes. For mequitazine $K \cdot k_{-1} = 1.67 \times 10^{-6}$ m $\cdot$ s^{-1} and $D_{org} = 1.17 \times 10^{-11}$ m$^2 \cdot$ s^{-1}, thus for mequitazine $1/K \cdot k_{-1} \gg 1/D_{org}$. Similarly for perphenazine $1/K \cdot k_{-1} \gg 1/D_{org}$. Therefore, for both phenothiazines, although the interfacial barriers are not rate-limiting, they are significant. The rate-limiting barrier to the mequitazine and perphenazine transport was therefore found to be the diffusion across IPM.

Mequitazine exhibited a larger interfacial resistance than perphenazine, and thus mequitazine was chosen to investigate further the effect of 1 : 1 ion-pairing with bile salts and how this influences interfacial transport. The influence of increasing sodium glycodeoxycholate concentration on the transport rate of mequitazine is depicted in Fig. 5. It is apparent that at low concentrations of bile salt the transport rate decreases slowly, possibly because of ion-pairing or complexation between cationic mequitazine and anionic glycodeoxycholate. However, at higher concentrations of sodium glycodeoxycholate, the transport rate is decreased markedly possibly due to the formation of mixed micelles of mequitazine–glycodeoxycholate. Barry and Gray (1975) have shown that quaternary ammonium compounds form mixed micelles with bile salts. In contrast, the partitioning of decamethonium bromide into *n*-octanol in the presence of bile salts has been reported to increase with increasing

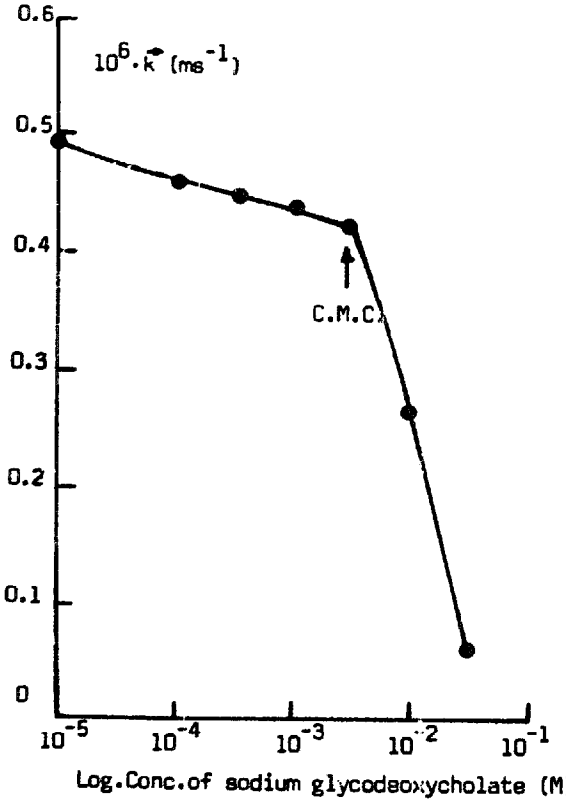

Fig. 5. The effect of bile salt (Na.GDC) concentration on the transport rate of mequitazine hydrochloride across IPM.

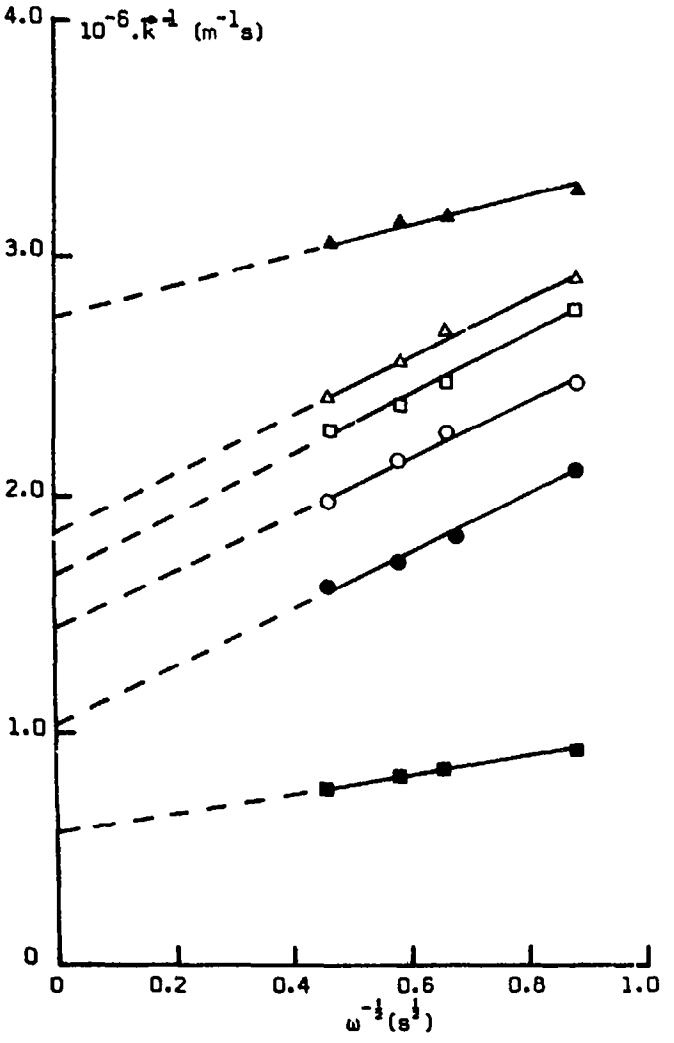

Fig. 6. Plot of $\vec{k}^{-1}$ against $\omega^{-1/2}$ for mequitazine hydrochloride permeating across IPM in the presence of bile salts at 37°C, pH 7.4. ●, mequitazine alone; ▲, Na.CiC + mequitazine; △, Na.GDC + mequitazine; □, Na.TDC + mequitazine; ○, Na.C + mequitazine; ■, Na.DC + mequitazine.

bile salt concentration, possibly due to lipid soluble ion-pair formation (Gaginella et al., 1974). The influence of sodium cholate, sodium deoxycholate, sodium taurodeoxycholate, sodium glycocholate and sodium glycodeoxycholate at a concentration of 1.4×10^{-4} M was examined on the transport of mequitazine. In man, the bile salts in concentrations up to 40 mmol · l^{-1} comprise about two-thirds of the solid matter in hepatic bile (Bell et al., 1976). Since bile salts form micelles at concentrations $> 1 \times 10^{-3}$ M (Small, 1971) the choice of 1.4×10^{-4} M concentration eliminates any contribution from micelle formation.

Fig. 6 shows typical plots of $\vec{k}^{-1}$ against $\omega^{-1/2}$ for mequitazine in the presence of the different bile salts. All the bile salts investigated decreased the transport of mequitazine across IPM except sodium deoxycholate. The reason for this anomalous increase is not apparent, but steric factors may be responsible. The decrease in

TABLE 2

THE EFFECT OF 1 : 1 ION-PAIRING OF VARIOUS BILE SALTS (1.4×10^{-4} M) ON THE INTERFACIAL TRANSPORT RATES OF MEQUITAZINE HYDROCHLORIDE (1.4×10^{-4} M) FROM AQUEOUS TO ORGANIC (k_{-1}) AND FROM ORGANIC TO AQUEOUS (k_1), AND ON AQUEOUS DIFFUSION COEFFICIENTS (D_{aq})

Bile salt	k_{-1} ($m\cdot Ms^{-1}$)	k_1 ($m\cdot Ms^{-1}$)	$D_{aq}\times10^{11}$ ($m^2\cdot s^{-1}$)
No bile salt	121.0	1.67	2.54
Sodium cholate	68.8	0.09	3.27
Sodium deoxycholate	259.0	3.58	15.03
Sodium taurodeoxycholate	57.6	0.79	3.01
Sodium glycocholate	32.0	0.44	10.64
Sodium deoxyglycocholate	53.8	0.74	2.84

transport by other bile salts, however, may be explained by the formation of unabsorbable, or slowly absorbable, ion-pairs or complexes. Table 2 lists interfacial transport rate constants, k_{-1} and k_1 of mequitazine in the presence of bile salts, together with aqueous diffusion coefficients of mequitazine in the presence of the same bile salts. Furthermore, there appears to be no relationship between the interfacial transport rate of mequitazine in the presence of bile salts and the corresponding association ion-pair constants determined conductometrically (Ahmed, 1981). It has been reported that bile salts at all concentrations appear to diminish the absorption of isopropamide, a quaternary ammonium compound, from the small intestine of the rat (Gaginella et al., 1974). The present in vitro data suggest that bile salts do not enhance the absorption of phenothiazine cations. Gaginella et al. (1974) have suggested that bile salts are of little physiological importance in the absorption mechanism of the highly water-soluble quaternary ammonium compounds. However, present in vitro data indicate that bile salts influence the transport of phenothiazines (tertiary amines) across membranes, but such in vitro data cannot be directly related to the in vivo situation.

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