J. Pharm. Pharmac., 1970, 22, 1–9 *Received June* 13, 1969

Some physico-chemical properties of SKF 525–A in aqueous solution

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The properties of solutions of SKF 525–A (β-diethylaminoethyl diphenylpropylacetate hydrochloride) have been investigated using surface tension, light scattering and microelectrophoretic techniques. The interaction of the drug with cholesterol and lecithin monolayers was studied by a surface balance method. SKF 525–A is more surface-active than chlorpromazine, and forms micelles containing 22 monomers in 0·9% NaCl solution. It increases the surface pressure of L-α-lecithin monolayers spread on water and both L-α-lecithin and cholesterol monolayers spread on 0·9% NaCl solutions at 1–2 × 10^{-6}M concentration levels. Because of the surface-active properties of SKF 525–A caution is urged in the interpretation of enzyme inhibition results as surfactants are well known as a class to greatly affect enzyme systems.

SKF 525–A (β-diethylaminoethyl diphenylpropylacetate hydrochloride) is an inhibitor of many microsomal enzymatic reactions (Brodie, Gillette & La Du, 1958) and thus prolongs the action of a variety of drugs. Brodie (1956) has suggested that its action may be to decrease the permeability of the microsomal membrane and so prevent or reduce the transport of drugs to the metabolic enzymes.

It has recently been reported (Lee, Yamamura & Dixon, 1968) that SKF 525–A is surface-active and behaves in a manner similar to chlorpromazine hydrochloride as far as it affects the haemolysis and stabilization of the red cell membrane. In order to gain further insight into the properties of the compound, the present investigation has been undertaken into its surface chemistry and micellar properties. Some comparisons have been made with the behaviour in solution of chlorpromazine and proniazid.

EXPERIMENTAL

Surface tension measurements were made using a drop volume apparatus incorporating an Agla micrometer syringe thermostatted at 25° ± 0·01°. The correction factors of Harkins & Brown (1919) were used. No significant ageing of the solutions was observed. *Viscosity measurements* were made in a suspended level viscometer at 25° ± 0·01°, the relative viscosities being referred to solutions at the critical micelle concentration (CMC). *Light-scattering* measurements were made at 25° with the light-scattering photometer described by Attwood (1968), on solutions of SKF 525–A in 0·9% and 2·5% NaCl which had been filtered through 0·2 μm Millipore filters. *Electrophoretic mobilities* were determined at room temperature (22° ± 1°) using a Zeta-Meter (Zeta Meter Inc.) with glass-Teflon cell. Octadecanol was dispersed in distilled water using an MSE ultrasonic generator and the resulting suspension of spherical particles diluted into solutions of drug of varying concentration. *Surface pressure measurements* at the air–water interface were made using a

glass Langmuir trough of 2·5 litre capacity employing a conventional torsion balance. For interaction studies the cholesterol or lecithin was spread from benzene on swept water surfaces and after the surface pressure-area curve had been obtained, 1 ml of an approximately 0·2% solution of the SKF 525–A or chlorpromazine was injected beneath the surface and the resultant surface pressure-area plot obtained. *pH measurements* were made using a Pye Model 78 pH meter by titration of the drug solution into water or salt solution.

Materials

SKF 525–A was a gift from Smith Kline and French Laboratories, Welwyn Garden City and was used as supplied (Proadifen HCl purity 99·8%). Chlorpromazine HCl (Largactil, May & Baker) was a commercial sample used as received. Iproniazid (Marsilid) was a gift from Roche Laboratories and used as received. L-α-lecithin (ex egg, grade II, Koch-Light Laboratories) was a 10% solution in hexane diluted to 0·1% with benzene for spreading. Cholesterol (BDH) was a dissolved in benzene. Octadecanol (melting point 58·3°), a purified sample, was a gift of Mr J. A. Rogers. NaCl was Analar quality. Water was once distilled from glass and twice distilled from potassium permanganate for surface tension studies. Whole blood diluted in drug solution was used for determination of erythrocyte electrophoretic mobilities.

RESULTS

SKF 525–A exhibits the properties of a typical ionic surface-active agent, lowering the surface tension of water and forming micelles above a certain critical concentration, the surface activity and micelle size increasing with addition of simple electrolytes.

Surface tension results shown in Fig. 1 indicate CMC's much higher than suggested by Lee & others (1968) being closer to 10^{-2}M rather than the value of 10^{-4} quoted by

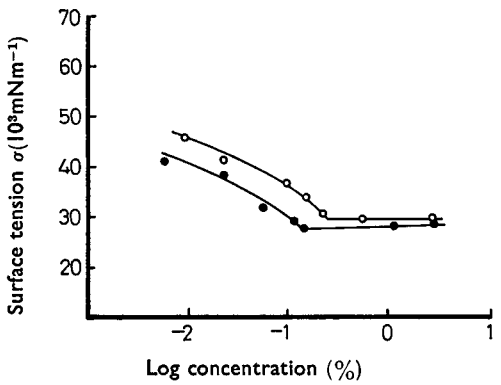

FIG. 1. Surface tension, mNm^{-1} (dynes cm^{-1}), plotted as a function of logarithm of concentration of SKF 525–A (%). ○ in 0·9% NaCl. ● 2·5% NaCl.

them, hence the onset of micellization is not connected with maximum erythrocyte membrane stabilization as they suggested, and cannot be biologically significant.

The limiting area/molecule of SKF 525–A, calculated from the surface tension values below the CMC, using the simple form of the Gibbs' equation, is 51 Å^2 (0·51 nm^2) in 0·9% NaCl which agrees well with the value of 53 Å^2 (0·53 nm^2) which was the area of the hydrophobic regions estimated from Catalin models. The surface tension of SKF 525–A at the CMC is lower than the corresponding values for chlorpromazine (Scholtan, 1955) indicating the greater surface activity of the former which probably results from the greater separation of the hydrophilic and hydrophobic regions in the SKF 525–A molecule.

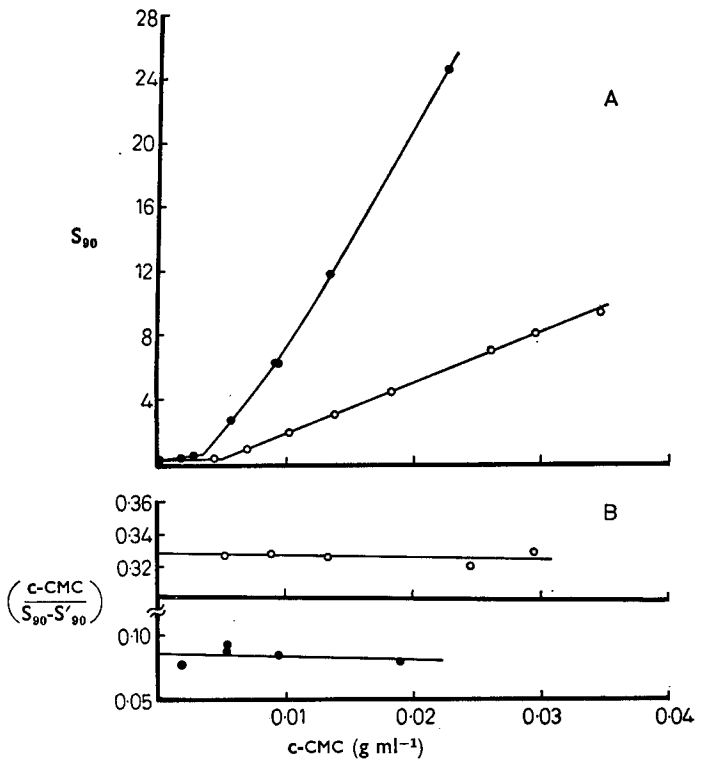

FIG. 2. A. The scattering ratio (S_{90}) as a function of concentration (g ml^{-1}) for SKF 525–A. ○ in 0·9% NaCl. ● 2·5% NaCl. B. Plots of (c–CMC)/(S_{90}—S_{90}) *vs* (c–CMC).

The high CMC's are confirmed by the light-scattering results which show distinct breaks in the scattering ratio (S_{90})–concentration (c) plots (see Fig. 2A). The light-scattering CMC's are higher than the surface tension CMC's. The latter are believed to be more accurate. Micellar weights were calculated from the reciprocal of the intercept of plots of (c-CMC)/(S_{90}–S'_{90}) *vs* (c-CMC) (Fig. 2B) which for charged surfactants in the salt solutions used is a valid procedure (Emerson & Holtzer, 1967). The micellar weights so obtained are shown in Table 1 along with CMC's determined by surface tension and light-scattering.

CMC's can also be obtained from pH *vs* log concentration plots (Lawrence & McDonald, 1957) and values from such measurements are also given in Table 1. This simple method is recommended as a rapid procedure to determine whether or

Table 1. *Limiting surface tensions, micellar weights and CMC's of SKF 525–A at 25°*

Salt concentration	Limiting σ (dynes cm^{-1}) or mNm^{-1})	Micellar weight*	Aggregation number	CMC M		
				by surface tension	by pH	by light-scattering
0	—	—	—	—	$9{\cdot}80 \times 10^{-3}$	—
0·9%	29·8	8,900	22	$5{\cdot}98 \times 10^{-3}$	$7{\cdot}88 \times 10^{-3}$	$1{\cdot}12 \times 10^{-2}$
2·5%	28·0	34,400	86	$3{\cdot}68 \times 10^{-3}$	$4{\cdot}38 \times 10^{-3}$	$8{\cdot}97 \times 10^{-3}$

* dn/dc value = 0·216, determined using a Hilger Rayleigh Interferometer at 25°.

not an ionic monobasic compound forms micelles, although the quantitative explanation of the shape of the resultant pH curves is not complete. With small micelles the CMC has to be interpolated (see Fig. 3). It is apparent from a qualitative treatment that the drug molecules are more highly dissociated in the micelle than in their free state below the CMC.

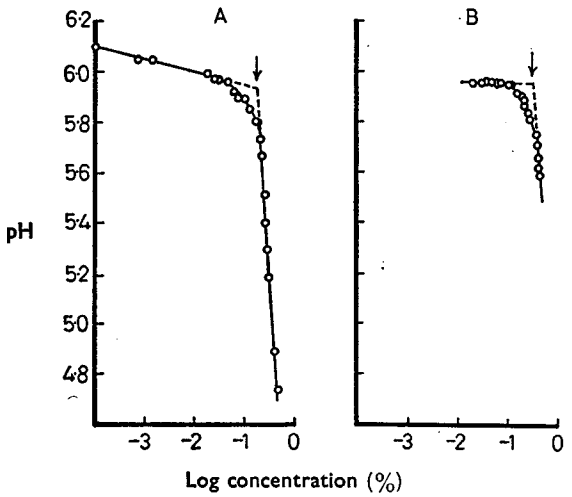

FIG. 3. Plots of pH *vs* log concentration (%) for SKF 525–A. A, in 2·5% NaCl; B, in 0·9% NaCl. The CMC is interpolated and shown by arrow. CMC values shown in Table 1 in M.

Fig. 4 shows the viscosity results plotted as reduced viscosity, $\eta_{sp}^{1}/(c\text{-CMC})$ *vs* (c-CMC). From these plots the viscosity intercept, $[\eta]$, was obtained. In both salt solutions, $[\eta]$ is 3·30 ml g^{-1} which indicates, on application of the viscosity equation, assuming spherical micelles

$$[\eta] = 2{\cdot}5\,(\bar{V}_2 + \omega V_1)$$

where $\bar{V}_2$ is the partial specific volume of the solute and V_1 of the solvent, an hydration, ω (g water/g drug) of 0·3, i.e., around 6 molecules of water per monomer which is of the same order as the hydration values of a variety of other ionic surfactants (Mukerjee, 1964).

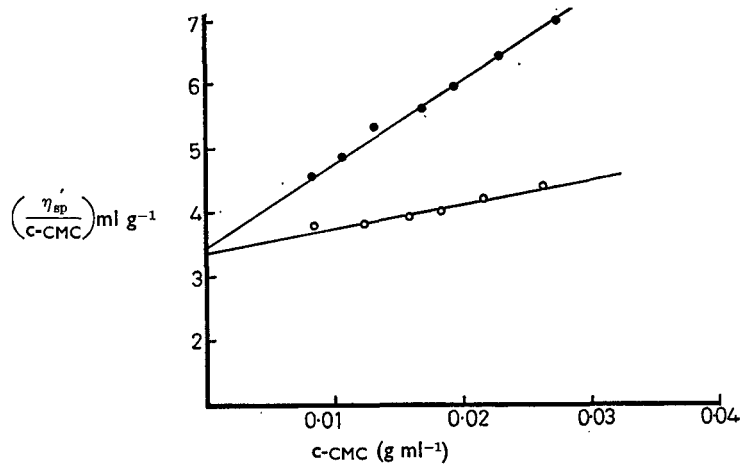

FIG. 4. Viscosity results at 25°C showing plots of $\eta_{sp}^1/(c\text{–}CMC)$ *vs* (c–CMC) in g ml^{-1}. ● SKF 525–A in 2·5% NaCl, ○ in 0·9% NaCl. Intercept, $[\eta] = 3{\cdot}3$ ml g^{-1}. η_{sp}^1 = (viscosity relative to CMC − 1).

Although more surface-active than chlorpromazine at the air–0·9% NaCl interface, SKF 525–A has a higher CMC and forms smaller micelles. The micellar weight of chlorpromazine is 20,400 in 0·9% NaCl, corresponding to 64 monomers per micelle; SKF 525–A forms micelles consisting of 22 monomers in the same salt concentration. The micellar size is strongly dependent on salt concentration, increasing to four times in 2·5% NaCl. This is still lower than the micellar weight of chlorpromazine which Scholtan (1955) records as 39,800 at this salt concentration.

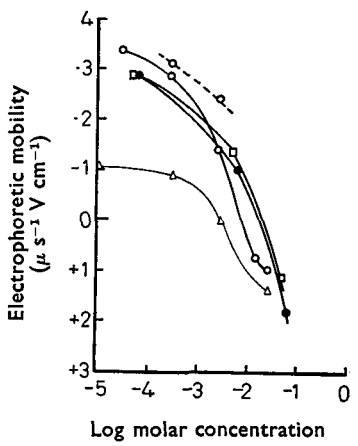

FIG. 5. Plots of electrophoretic mobilities of octadecanol in the presence of: ○ SKF 525–A, ● iproniazid, □ chlorpromazine hydrochloride. Dotted line represents results obtained by addition of HCl to the dispersion. The lowering of electrophoretic mobility caused by the drugs is more marked. △ Effect of SKF 525–A on electrophoretic mobilities of human erythrocytes.

The microelectrophoresis experiments were made to determine whether extensive adsorption of SKF 525–A at solid interfaces could be detected at very low drug

concentrations. Octadecanol was chosen as the adsorbent as it has been used as a model for a biological interface (Hollingshead, Johnson & Pethica, 1965). The sample of octadecanol dispersed in deionized water had an electrophoretic mobility of $-4{\cdot}3\,\mu s^{-1}$ $(V\,cm^{-1})^{-1}$ $(-4{\cdot}3 \times 10^{-8}\,m^2\,V^{-1}\,s^{-1})$. The adsorption of the positively charged SKF 525–A and chlorpromazine HCl can be followed in Fig. 5 by the reduction in the negative charge of the octadecanol particles. Reversal of charge occurs at $3{\cdot}96 \times 10^{-3}$M for SKF 525–A and $1{\cdot}12 \times 10^{-3}$M for chlorpromazine HCl. In both cases there is little effect on electrophoretic mobility below 10^{-5}M.

Electrophoretic mobilities of human erythrocytes in distilled water were found to be $-1{\cdot}1\,\mu s^{-1}$ $(V\,cm^{-1})^{-1}$ $(-1{\cdot}1 \times 10^{-8}\,m^2\,V^{-1}\,s^{-1})$ in good agreement with those quoted by Abramson, Moyer & Gorin (1942). SKF 525–A reduced the mobility of the erythrocytes, reversing their charge at $2{\cdot}5 \times 10^{-3}$M (see Fig. 5). These experiments were made in the absence of added salt and it is probable that in the presence of NaCl a greater lowering of electrophoretic mobility would be noted. Below 10^{-3}M SKF 525–A results in stabilization of the red cell membrane and above this concentration haemolysis ensues (Lee & others, 1968), SKF 525–A maintaining its ability to stabilize the membrane at concentrations as low as 10^{-9}M (Lee & others, 1968). This is surprising in view of the fact that the compound does not lower the surface tension noticeably until 10^{-6}M nor the electrophoretic mobility of the erythrocytes until concentrations of 10^{-4}M have been reached.

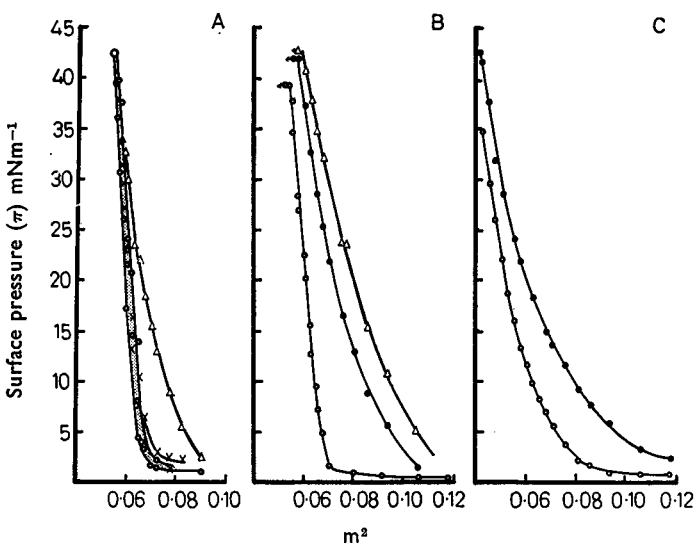

FIG. 6. Surface pressure, π (mNm^{-1}) *vs* area occupied by 10^{-3} g of film (m^2). A: ○ cholesterol film alone on water; × with $17{\cdot}6 \times 10^{-4}$ g $litre^{-1}$ SKF 525–A injected beneath the monolayer; △ with a total of $35{\cdot}5 \times 10^{-3}$ g $litre^{-1}$ SKF 525–A in substrate. B: ○ Cholesterol film alone; ● $17{\cdot}6 \times 10^{-3}$ g $litre^{-1}$ SKF 525–A after 1 h 40 min, △ total of $35{\cdot}5 \times 10^{-4}$ g $litre^{-1}$ SKF 525–A after 1 h. C: ○ Lecithin monolayer alone; ● with $17{\cdot}6 \times 10^{-4}$ g $litre^{-1}$ SKF 525–A injected beneath the monolayer. In Figs 6–7 stippled area indicates accuracy with pure monolayers.

The penetration of lipid monolayers by psycho-active drugs (Zografi & Auslander, 1965; Demel & van Deenen, 1966) and by local anaesthetics (Skou, 1961) has been studied in the belief that this should give some indication of the site of action of the drugs and an understanding of the mode of interaction of the drugs with the lipids.

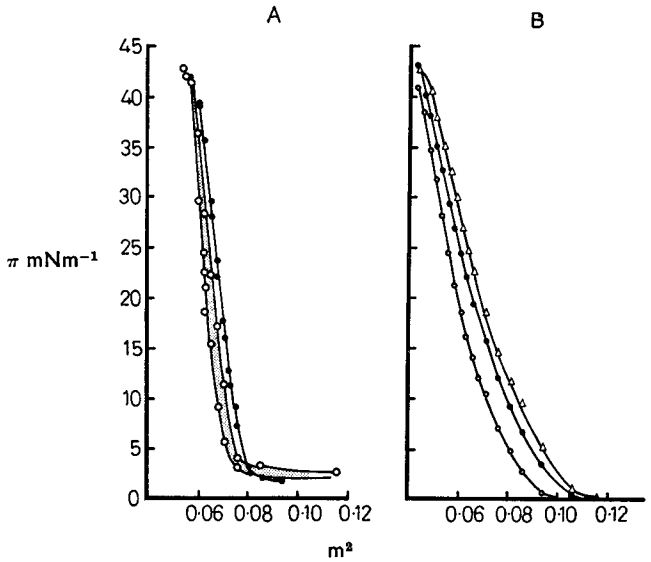

FIG. 7. A. ○ Cholesterol monolayer spread on water, ● with $17{\cdot}6 \times 10^{-4}$ g litre^{-1} chlorpromazine hydrochloride in substrate. B. ○ Lecithin monolayer spread on 0·9% NaCl; ● $17{\cdot}6 \times 10^{-4}$ g litre^{-1} chlorpromazine hydrochloride injected beneath the monolayer; △ with $35{\cdot}5 \times 10^{-4}$ g litre^{-1} chlorpromazine hydrochloride.

Because the membrane of the microsome has been implicated by Brodie (1956) as a possible site of action of SKF 525–A, studies were made with monolayers of cholesterol and lecithin spread on water and salt solutions beneath which had been injected SKF 525–A and chlorpromazine. Some results are shown in Figs 6 and 7 and Table 2. On water, SKF 525–A has no effect on cholesterol films and chlorpromazine has a limited effect (both drugs at 2×10^{-5}M). At 6×10^{-5}M SKF 525–A there was evidence of some interaction (Fig. 6). When the substrate was 0·9% NaCl much more pronounced interaction was noted between SKF 525–A and the cholesterol. On water, however, SKF 525–A interacts with the lecithin monolayer.

Table 2. *Increases in surface pressure and area caused by penetration into lecithin and cholesterol monolayers*

Compound	Monolayer	Substrate	Area at π = 30 mNm^{-1} (Å^2 = 10^{-20}m^2)*	Increase in surface pressure, at A = 0·06 m^2, dynes cm^{-1} or mNm^{-1}
SKF 525–A	Cholesterol	H_2O	37·3 Å^2 (37·9)	0
	Cholesterol	0·9% NaCl	41·2 Å^2 (37·2)	14·3
	Lecithin	H_2O	78·5 Å^2 (61·45)	12·0
	Lecithin	0·9% NaCl	65·1 Å^2 (57·3)	8·6
Chlorpromazine	Cholesterol	H_2O	41·75 Å^2 (39·8)	5
	Lecithin	0·9% NaCl	70·31 Å^2 (66·4)	5·9
Iproniazid	Cholesterol	H_2O	41·6 Å^2 (39·8)	3·0
	Lecithin	H_2O	66·7 Å^2 (61·5)	4·8

* Figures in brackets are the areas/molecule of the monolayer molecules before addition of drug.

Table 2 lists some of the results of the film penetration studies, showing the increases in surface pressure at a particular area/molecule of the original film and changes in apparent area/molecule at a surface pressure of 30 dynes cm^{-1} (mNm^{-1}). The results, in general, follow the trends observed by Zografi & Auslander (1965) in that penetration into the liquid expanded films of lecithin appears to be easier than into the condensed cholesterol films.

The penetration of both chlorpromazine and SKF 525–A into the cholesterol films changes the character of the monolayers from a condensed to a liquid expanded film (Figs 6 and 7).

Some work was done with iproniazid as it has been pointed out (Brodie, 1956) that although structurally unrelated to SKF 525–A, iproniazid inhibited the same enzyme systems as the latter. A 1% solution has a surface tension of 48 mNm^{-1} and interacts with lecithin and cholesterol monolayers to a small extent (Table 2).

In some cases (e.g., the lecithin-SKF 525–A system) the surface pressure increased over a long period (see Fig. 6). This is perhaps due to the low concentration of drug and the finite mixing time of the 1 ml of drug solution with the substrate liquid. It is not considered to be significant biologically.

DISCUSSION

The ability of SKF 525–A to lower surface tension is pronounced, the limiting surface tension being of the order of 29 mNm^{-1} which is comparable to the lowering of surface tension produced by many conventional surface-active agents. The CMC in water and saline is too high to be of biological significance, but the ability of the SKF 525–A molecules to penetrate cholesterol and lecithin monolayers at concentrations around 2×10^{-6}M is likely to be of importance in view of the proposed modes of action of the drug on the microsomal membrane. The difficulty however is to decide whether surface activity is simply incidental, i.e., the result of the amphipathic structure of the drug molecule or whether it is vital to the function of the drug (Florence, 1968). As most ionic surface-active molecules will penetrate spread insoluble monolayers it has also to be decided whether such interaction is of biological significance. As the drug affects the duration of activity of a number of drugs such as hexobarbitone it is reasonable to presume that SKF 525–A action is one on the microsomes either preventing the entry of the drug into the microsome, as Brodie suggests, or by an inhibitory effect on the particular enzyme involved. *In vitro* studies on enzyme systems are unlikely to answer this question as many ionic surfactants which are biologically inactive are known to inhibit enzyme systems, i.e., enzyme inhibition might simply be a by-product of the surface activity and is perhaps non-specific.

Adsorption of the positively charged SKF 525–A molecules at low concentrations does not reverse the charge on the surface of octadecanol or human erythrocytes. But in both cases the surface is not made positive. It is unlikely, therefore, that physical adsorption of SKF 525–A on the microsomal surface will act as a physical barrier repelling drugs of opposite charge, at low concentrations.

The penetration results show that the SKF 525–A molecule is capable of interacting with the components of cell membranes. Chlorpromazine, which has none of the enzyme inhibitory properties of SKF 525–A, also interacts with these components. Demel & van Deenan (1966) have shown that orphenadrine hydro-

chloride, which when given to rats *enhances* the inactivation of some drugs (Remmer, 1962), interacts with cholesterol monolayers but not significantly with lecithin spread on water.

Further work is necessary, perhaps with membrane diffusion cells, to determine whether the drugs alter the permeability of membranes in a specific way.

Acknowledgements

I thank Smith Kline and French Laboratories for the gift of the SKF 525–A, Roche Ltd., for the Marsilid sample and Mrs Christine Selkirk for technical assistance.

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