J. Pharm. Pharmac., 1975, **27**, 152–159 *Received June* 21, 1974

Non-ionic surfactants and membrane transport of thioridazine in goldfish

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Polysorbate 80, which has been widely used in studies of the effects of surfactants on drug absorption, increases the rate of absorption of some drugs at concentrations near its critical micelle concentration (cmc). To determine whether all non-ionic surfactants were capable of inducing this effect, the effects of six commercial non-ionic surfactants on thioridazine absorption in goldfish have been compared with the effect of polysorbate 80. The reciprocal death time (T^{-1}) determined when the fish were immersed in the solution under study was the index of absorption rate used. Not all surfactants tested increased T^{-1}. Cremophor EL (polyoxyethylated castor oil), Atlas G1295 (a polyoxyethylene fatty glyceride), Atlas G1300 (a polyoxyethylene glyceride ester) had no effect below their cmc's. Those surfactants that did increase T^{-1} [polysorbate 80 (a polyoxyethylene sorbitan monooleate), Atlas G1790 (a polyoxyethylene lanolin derivative), G2162 (a polyoxyethylene oxypropylene monostearate) and Renex 650 (a polyoxyethylene alkyl aryl ether)] display the concentration-dependent behaviour reported previously—a decrease in absorption rate when the surfactant concentration is increased above its cmc. The factor determining whether or not the surfactant will increase absorption rate appears to be the configuration of the surfactant molecule rather than its hydrophile-lipophile balance or its surface activity.

Non-ionic surfactants can increase the absorption of drugs from solution (Elworthy, Florence & Macfarlane, 1968; Gibaldi & Feldman, 1970) enhance the activity of antibacterial agents (Saski & Shah, 1965; Brown & Winsley, 1969, 1971) or increase the penetration of herbicides into leaves (Becher & Becher, 1969). These effects occur when the surfactants are present below their critical micelle concentration (cmc). Above this concentration there is, generally, a decrease in absorption or antibacterial efficacy due to solubilization or binding of the active species within or onto the surfactant micelles.

Whether all non-ionic surfactants had the ability to increase absorption rates was not obvious. Nor was it clear by what mechanism those surfactants which increased transport across membranes did in fact exert this action. There is evidence that where permeability is increased a specific effect on the lipid membrane is involved (Cuthbert, 1967). It is likely that penetration of the non-ionic surface-active agent into the lipid layers of the membrane increases permeability, acting as it were like a plasticizer of the hydrocarbon lipid chains in the membrane interior.

In this paper we set out some results of experiments designed to show whether or not absorption or transport promotion was a common characteristic of non-ionic

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surfactants and to determine more about the mechanism of action of the active surfactant compounds. Not all the surfactants tested by us increased the absorption of thioridazine hydrochloride in our test system—the goldfish—thus an attempt has been made to relate surfactant structural and physical parameters with this activity.

MATERIALS AND METHODS

Goldfish (*Carassius aurata*) were used as before (Florence, 1970) in 200 ml of drug solution. The death time of 5 fish weighing from 2·5 to 3·5 g was determined and the mean death time (T) calculated. Following Levy & Gucinski (1964) the mean reciprocal death time (T^{-1}) was used as an index of drug absorption. The kinetics of drug absorption in goldfish has recently been studied in some detail by Yalkowsky, Carpenter & others (1973) who obtain an equation

$$1/T = k_1 C_B/C_F - k_2/2$$

where C_B and C_F are the concentrations in the bathing solution and the threshold concentration in the fish respectively, and k_1 and k_2 are the rate constants of passive absorption and elimination of the drug. Comparative reciprocal death times in solutions of identical drug concentration should therefore reflect changes in k_1 and k_2 provided that the intrinsic drug activity (i.e. C_F) is unaltered by the additive.

The non-ionic detergents except those named below (see Table 1) were obtained from Honeywill-Atlas, Carshalton, Surrey and were used as received. Cremophor EL was obtained from Badische-Anilin. Pluronics F38, F88 and P123 were samples obtained from Ugine Kuhlmann (Chemicals) Ltd., Middlesex. All the surfactants used were tested for lack of toxic effects on goldfish at concentrations higher than those used in this work.

Thioridazine hydrochloride was a gift from Sandoz Ltd. Cholesterol was BDH Biochemical grade material.

Surface tension measurements were made at room temperature (20°) using a glass Wilhelmy plate (4·8 cm perimeter) and a torsion balance (0 to 250 mg, White Instruments Ltd.). Critical micelle concentrations were obtained from plots of surface tension (σ) versus logarithm of concentration (c) and limiting areas per molecule (A) of the adsorbed surfactants were calculated using the simple form of the Gibbs' equation to calculate the surface excess concentration, Γ (mol m^{-2}).

$$\Gamma = -\frac{1}{2{\cdot}303RT}\left(\frac{d\sigma}{d\log c}\right) \quad .. \quad .. \quad .. \quad (1)$$

where $\Gamma = 1/A\ N$, N being Avogadro's number.

Surface pressure measurements were made using a Langmuir trough constructed of glass and a torsion balance with glass (4·8 cm perimeter) Wilhelmy plate. Films of cholesterol were spread from solutions in Analar benzene onto water or dilute detergent solutions.

Viscosity measurements were made in a suspended level dilution viscometer at 25 ± 0·01°. The intrinsic viscosity, $[\eta]$ (see Table 1), was obtained from plots of η_{sp}/C^* where C^* is the micellar concentration and η_{sp} is $\eta_{rel} - 1$.

Binding of thioridazine to the non-ionic surfactants was determined by a non-equilibrium dialysis technique. Two 10 ml capacity glass half-cells were separated by a Visking cellophane membrane. Drug-surfactant solution was placed in one cell,

solvent in the other and the rate of transport of the drug into the recipient cell determined over 2 h during which time the increase in concentration with time was linear. The cells were rotated at 24 rev min^{-1}. The rate of dialysis (r) was considered to be proportional to the concentration of free drug present, the percentage drug bound being calculated from 100 r_s/r_o, the subscripts s and o referring to surfactant and pure water systems respectively.

RESULTS AND DISCUSSION

Effect of pre-micellar concentrations of the surfactants

Fig. 1 shows the effects of the various surfactants on the reciprocal death times of fish immersed in 0·04% thioridazine hydrochloride. The reciprocal death time is proportional to the rate of absorption k_1 (Nightingale & Gibaldi, 1971) and the results we obtained in this experiment therefore reflect changes in the rate of absorption of drug, if the rate of exosorption is low. Polysorbate 80 (Tween 80), Renex 650, Atlas G1790 and Atlas G2162 increase absorption at low concentrations and also exhibit the characteristic decrease in drug absorption at surfactant concentrations higher than the cmc (Levy, Miller & Reuning, 1966; Florence, 1970). In each case increased absorption rates occurred at pre-micellar concentrations. Therefore in some way it is the onset of micelle formation which prevents further increases in k_1.

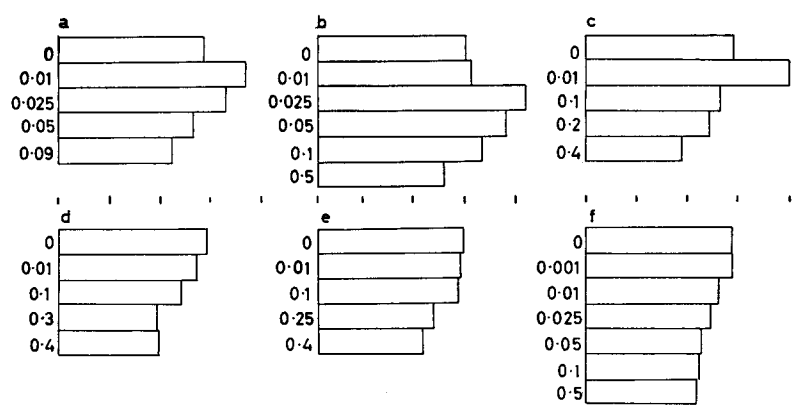

FIG. 1. Examples of the effect of surfactant concentration (% w/v) on mean reciprocal death times of goldfish, shown on the abscissa in units of 0·02 min^{-1}. (a) G2162, (b) Renex 650, (c) G1790, (d) G1298, (e) G1300, and (f) Cremophor EL. (d), (e) and (f) show no evidence of potentiation of activity.

Cremophor EL, Atlas G1295 and G1300 do not increase the activity of the thioridazine at any concentration studied.

Complex relations between the hydrophile-lipophile balance (HLB) of non-ionic detergents and their ability to increase the activity of herbicides (Becher & Becher, 1969) and to increase corneal permeability (Marsh & Maurice, 1971) have been observed. No simple relation can be detected in our results (Table 1), nor is the ability to increase drug absorption a function of surface tension.

Examination of the data seems to indicate that the molecular size and shape of the surfactant rather than its HLB is important in determining whether it has the ability to increase the permeability of the gill membranes. Those surfactants that are not active, Atlas G1295 and G1300 have three very large ethylene oxide residues. Where

Table 1. *Some properties of the non-ionic surfactants.*

Surfactant	Type and (monomer molecular weight)*	HLB	cmc (%) w/v	Area/ molecule (10^2nm^2)	Limiting surface tension (mN m^{-1})	[η] ml g^{-1}	Approximate concentration for maximum enhancement (%)
Tween 80 (polysorbate 80)	Polyoxyethylene sorbitan mono-oleate (1570)	15·0	0·01	76	42·7	5·55	0·01
Renex 650	Polyoxyethylene alkyl aryl ether (1010)	17·1	0·04	126	45·1	8·61	0·025
Atlas G 1790	Polyoxyethylene lanolin derivative (1350)	11·0	0·01	178	42·4	7·35	0·01
Atlas G 2162	Polyoxyethylene oxypropylene monostearate (990)	16·0	0·025	166	41·2	6·90	0·01
Cremophor EL	Polyoxyethylene castor oil (1680)	13·3	0·02	95	42·0	5·65	None
Atlas G 1295	Polyoxyethylene fatty glyceride (3450)	17·5	0·28	189	43·6	12·7	None
Atlas G 1300	Polyoxyethylene glyceride ester (1460)	18·1	0·22	146	43·3	13·7	None

* Monomer molecular weight determined in benzene by osmometry by Department of Pure and Applied Chemistry, University of Strathclyde.

the surfactant has several long hydrophilic chains rather than a single ethylene oxide residue or several shorter chains, drug absorption will not be increased. It is possible that those surfactants with multiple chains are prevented from interacting intimately with the lipid membrane because of their bulk. In an attempt to elucidate this further, the effect of three Pluronic surfactants (F38, F88 and P123) was studied, the molecular weights of which were respectively 5000, 10 800 and 5650. Pluronic F38 and F88 are both 80% hydrophile, while Pluronic P123 was 30% hydrophile. F38 and F88 had no effect on thioridazine absorption, while P123 increased absorption. This would indicate that the greater the hydrophilic nature of the molecule the less active it is in promoting absorption and this would be in agreement with the results with Atlas G1295 and 1300. However, size alone cannot be the sole criterion, as Pluronic P123, which has a molecular weight of over 5000, increased T^{-1}.

Monolayer results

To determine whether there was a quantitative difference in the penetration of lipid monolayers by the surfactants, cholesterol was spread upon solutions of very low concentrations of the surfactants (6×10^{-7} M except for G1295 and G1300 solutions

which were 6×10^{-6} M). Increase in surface pressure (Π) at an area of 0·4 nm² (area per molecule of cholesterol spread on water) was taken as evidence of monolayer penetration. For convenience the surface pressure at 0·5 nm² was compared. Surface pressures of the systems are shown in Table 2. There appears to be some significant differences in the ability of the surfactants to penetrate the monolayer which correlates with the ability or otherwise to increase permeability. At higher surfactant concentrations solubilization of cholesterol occurs from the surface. Apart from the result for Cremophor EL there is a good correlation between the molar cmc of the surfactants and their ability to penetrate the cholesterol monolayer (Table 2).

Table 2. *Surface pressures of cholesterol monolayers at 0·5 nm² and critical micelle concentrations* (M) *of the surfactants.*

Surfactant	Tween 80	G 1790	G 2162	Renex 650	Cremophor EL	G 1295	G 1300
Surface pressure Π(mN m^{-1})	13·5	14·5	12·0	11·3	9·5	9·8	6·8
Cmc (10^4 M)	0·64	0·74	2·53	3·96	1·19	8·12	15·1
Effect on absorption	+	+	+	+	—	—	—

Effect of micellar concentrations of surfactant

The decrease in reciprocal death time at surfactant concentrations above the critical micelle concentrations may be due to several factors. The decreased chemical potential of the drug due to formation of mixed micelles (or to the solubilization of the drug) will tend to decrease partitioning of drug into the fish. The increased viscosity of the system is not great and can be neglected in this discussion. Physical blocking of the membrane surface by the adsorbed surfactant may prevent access by the drug. A complicating factor in those cases where enhancement of absorption

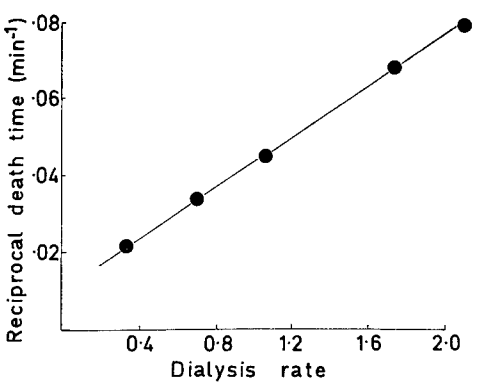

FIG. 2. Relation between reciprocal death time (min^{-1}) of goldfish and the rate of dialysis of thioridiazine hydrochloride from solutions of varying concentrations. Both reciprocal death time and rate of dialysis are linear functions of free drug concentrations over the range studied. The points shown here are from smoothed plots of these two functions.

occurs is that it is possible that at post-cmc concentrations, a dual effect is being observed, i.e. permeability is increased above normal but there is a simultaneous decrease in transport due to drug-micelle interaction, or to blocking effects. It is therefore easier to first consider the decrease in T^{-1} in those systems where no increase in activity has been exhibited.

There is a linear relation between reciprocal death times and rate of dialysis of thioridazine through Visking membranes in the range of drug concentrations of interest, (Fig. 2) which suggests that the dialysis experiment is valid in assessing the effect of drug-micelle interaction on drug absorption. Fig. 3 shows the dialysis rate of the drug in increasing concentrations of polysorbate 80 and Cremophor EL. Similar results were obtained with the other detergents studied. Within experimental error there is no increase of dialysis *in vitro* at low detergent concentrations. In the absence of biological interactions with the gill membrane, the decreasing rate of dialysis *in vitro* should be reflected in the decrease in T^{-1}. The results for the "inactive" surfactants are shown by the open symbols in Fig. 4. (The solid symbols give the results for the absorption promoting surfactants. Most points clearly show the increased permeability of the membrane by their position above the line representing the results obtained with thioridazine alone). The results would seem to indicate

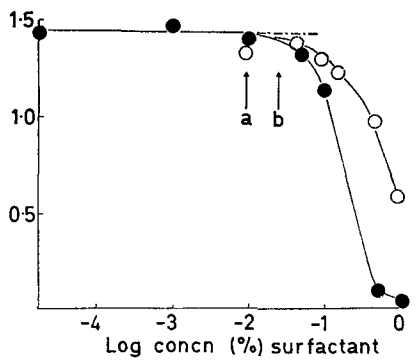

FIG. 3. Rates of dialysis (arbitrary units) of thioridazine hydrochloride in the presence of ● polysorbate 80 and ○ Cremophor EL. The arrows represent the critical micelle concentrations of the surfactants; a, polysorbate; b, Cremophor.

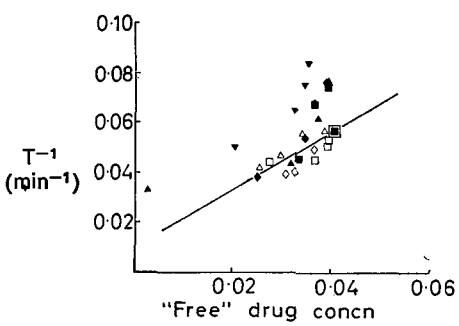

FIG. 4. A plot of reciprocal death time of goldfish against free drug concentration determined from the dialysis rate experiments. Open symbols give results for the death times in solutions containing non-ionic surfactants which do not increase absorption at pre-micellar concentrations. Solid symbols represent results obtained with solutions containing the absorption promoting surfactants. □ Cremophor EL, △ G1300, ◇ G1295, ▲ Tween 80, ▼ Renex 650, ■ G2162, ◆ G1790. Solid line obtained from results in Fig. 2.

that for Cremophor EL, for example, its adverse effect on thioridazine absorption can be ascribed to the decrease in free thioridazine. It is likely that no solubilized drug is transported across either of the membranes under consideration (Mysels, 1969). However, at the highest concentrations of Cremophor EL and G1300 studied, correction of the reciprocal death times to account for bound drug results in higher values than those in absence of surfactant. This suggests that as suspected at these high concentrations some membrane integrity is lost and that the resulting increase in permeability is masked by binding of the drug to the surfactant aggregates, the overall effect observed being a reduction in T^{-1}. Results for G1300 and Tween 80, however, are not as readily interpreted. In intermediate concentrations between the cmc and the highest concentration, the binding of the drug to these detergents does not account for the extent of decrease in T^{-1} (Fig. 5) and one must invoke some blocking mechanism.

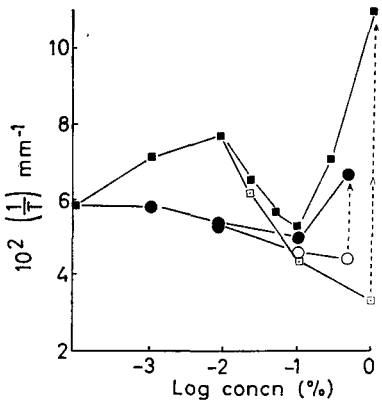

FIG. 5. Reciprocal death times of goldfish as a function of the logarithm of concentration of polysorbate 80 (□ ■) and Atlas G1300 (○ ●). Open circles experimentally determined values (as shown in Fig. 1). Filled circles values after correction for solubilization or mixed micelle formation according to dialysis data. Dotted lines show largest corrections in the system.

Although Heckmann's theory of single-file diffusion (Heckmann, 1972) was constructed on the basis of diffusion through pores, it is tempting to apply it in an attempt to explain results obtained in our systems. Of particular interest are the predictions that Heckmann makes of the net flux of one molecular species in the presence of another which also diffuses across the membrane. A schematic representation of the predicted effects when two species diffuse along pores which are several molecular diameters long is shown in Fig. 6. This shows the flux (ϕ) of species 1 (e.g. drug) as a function of ΔC_2 the concentration gradient of species 2 (e.g. surfactant). This results from the effect of the competition of drug and surfactant molecules for sites inside the pore. Heckmann suggests that if a system shows this behaviour it is not only possible that single file diffusion is the cause but that it is possible that a more or less 3-D network of sites is present. We suggest that if surfactant molecules penetrate between the lipid chains of the absorbing membrane, the presence of a sufficiently high number of the surfactant molecules will block entry of drug molecules so that their net flux decreases. This would apply to both classes of surfactant—those which do and those which do not increase absorption, and may well explain why correction for interaction of drug with micelles does not explain the results for polysorbate 80 or for Atlas G1300 as illustrated in Fig. 5. Just above the cmc the

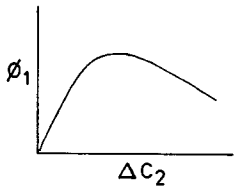

FIG. 6. Flux of component 1 (ϕ_1) as a function of the gradient of concentration (ΔC_2) of component 2 across a membrane according to Heckmann (1972).

measured binding is insufficient to explain the total decrease in activity yet at the highest concentrations studied, binding corrections will overcompensate. One must assume that just above the cmc blocking occurs readily because of the penetration of the surfactant. Those surfactants which increase absorption most would be most readily taken inside the membrane. At higher concentrations membrane integrity is lost—indicated by the dramatic upsurge in the "corrected" value of T^{-1}.

Preferential uptake of the unionized species by the non-ionic micelles would lead to an under-estimate of the required correction for the transport results. At the pH of the surfactant-drug solutions, however, almost all the drug is in the ionized form. In addition Levy & Anello (1968) could find no difference in the effect of polysorbate 80 on the absorption of non-ionized or ionized forms of secobarbitone by goldfish.

We conclude that some surfactants because of their configuration and hydrophilic properties do not increase the absorption of thioridazine across the gill membrane of goldfish, although at high concentrations membrane integrity is affected. The surfactants able to increase permeability do so at low concentrations by penetrating into the lipid portion of the membrane. In all cases the decrease in reciprocal death times above the cmc is caused by the interaction of drug with micelles, but some surfactants also decrease the access of drug into membrane just above the cmc before sufficient surfactant has entered to decrease the resistance of the membrane at high bulk concentrations.

Acknowledgements

J.M.N.G. was the recipient of the Wellcome Award of the Pharmaceutical Society of Great Britain for 1970–1973.

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