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Non-ionic surfactants and the membrane transport of barbiturates in goldfish *

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Summary

The effect of a series of polyoxyethylene non-ionic surfactants on the membrane transport of barbiturates in goldfish has been assessed using the overturn time technique. The surfactants, ranging in polyoxyethylene chain length from 2 to 60 and alkyl chain length from 4 to 18, were used at concentrations ranging from 0.001 to 0.1% w/v. Presence of the surfactants in the bathing medium caused a concentration-dependent alteration in the absorption of secobarbitone and thiopentone. The greatest enhancement of absorption for each barbiturate occurred with surfactants with 10 to 20 ethylene oxide moieties, alkyl chain lengths of $C_{12}-C_{16}$ and molecular areas of between 1.00 and 1.60 nm^2.

The results are comparable with data obtained with the same surfactants using other epithelial membranes.

Introduction

Surfactants are major components of pharmaceutical and cosmetic formulations because the unique solution properties of these substances result in their widespread use as emulsion and suspension stabilizers, wetting agents and detergents (Schick, 1967). The principal determinant of these properties is the ability of the surfactant to adsorb at interfaces (Rosen, 1978) leading to a reduction in interfacial tension. In biological systems the effect of surfactants is complex particularly their effect on cell

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membranes which can lead to alterations in permeability patterns (Gibaldi and Feldman, 1970; Levy et al., 1966; Anello and Levy, 1969; Florence and Gillan, 1975; Zaslavsky et al., 1978; Whitmore et al., 1979). Most of the literature on this subject concerns the effects of one or two surfactants on the rate of permeability of drugs and it is difficult to discern the properties of surfactants that result in enhanced membrane permeability. An attempt has been made to relate the physicochemical properties of surfactants to their ability to enhance absorption in the goldfish (Florence and Gillan, 1975), but in a variety of surfactants, ranging in hydrophile–lipophile balance (HLB) from 13.3 to 18.1 and in molecular weight from 990 to 10,800, no relationship between increase in absorption and surfactants HLB was observed.

Previous investigations with polyoxyethylene non-ionic surfactants have demonstrated that in order to enhance gastric mucosal permeability of paraquat a definite range of both alkyl and polyoxyethylene chain length is required (Walters et al., 1981). In order to expand these studies to include permeants with a wider range of physicochemical properties the effect of several polyethoxylated non-ionic surfactants on barbiturate absorption in goldfish has been investigated.

In an attempt to gain some understanding of the complexities arising from surfactant–permeant interaction the barbiturates were selected to provide a wide range of permeant lipophilicity. Goldfish have been used widely in membrane transport studies mainly because they provide a convenient uncomplicated membrane system, are easily handled and are readily obtainable. A complication in this type of study, however, is the intrinsic biological (anaesthetic or toxic) activity of some non-ionic surfactants to goldfish (Florence et al., 1978); the surfactants discussed here, however, have negligible biological activity.

Materials and Methods

Goldfish (*Carassius auratus*) were obtained locally. Only fish weighing between 2.5 and 5.0 g were used and they were allowed to acclimatize for 48 h after delivery.

Thiopentone sodium was obtained from Abbot Laboratories, Queenborough, Kent. Secobarbitone sodium was obtained from Sigma London Chemicals, Poole, Dorset. The surfactants (Table 1) were those obtained and used previously (Walters et al., 1981).

All reagents were used as supplied. Standard 1% aqueous solutions of the surfactants were used. For those surfactants with short ethylene oxide chains uniform cloudy dispersions were obtained on heating. All aqueous solutions were made using distilled water.

The drugs used in this study were 3 barbiturates with similar dissociation constants but widely different oil/water partition coefficients. Phenobarbitone has a pK_a at 25°C of 7.3 and a methylene chloride/water partition coefficient, P, of 3. Secobarbitone has a pK_a of 7.9 and $P = 52$ while thiopentone has a pK_a of 7.4 and $P = 580$ (Bush, 1963).

TABLE 1

SURFACE PROPERTIES OF POLYOXYETHYLENE NON-IONIC SURFACTANTS

Surfactant	Type	HLB	Cmc (% w/v)	Area/molecule (nm^2)
Brij 30	POE [a] (4) lauryl ether	9.7	0.006	0.38
Brij 35	POE (23) lauryl ether	16.9	0.01	1.15
Brij 36T	POE (10) lauryl ether	15.2 [c]	0.008	1.09
Brij 52	POE (2) cetyl ether	5.3	0.003	0.27
Brij 56	POE (10) cetyl ether	12.9	0.006	1.10
Brij 58	POE (20) cetyl ether	15.7	0.01	1.48
Brij 72	POE (2) stearyl ether	4.9	0.002	0.30
Brij 76	POE (10) stearyl ether	12.4	0.005	1.06
Brij 78	POE (20) stearyl ether	15.3	0.009	1.54
Brij 92	POE (2) oleyl ether	4.9	0.002	0.29
Brij 96	POE (10) oleyl ether	12.4	0.006	1.03
Brij 98	POE (20) oleyl ether	15.3	0.01	1.51
Myrj 52	POE (40) stearate	16.9	0.03	2.34
Texofor A6	POE (6) cetyl ether	5.3	0.002	0.60
Texofor A14	POE (14) cetyl ether	14.4	0.007	1.20
Texofor A60	POE (60) cetyl ether	18.3	0.06	2.56
–	POE (20) butanol	18.8 [c]	–	1.15
–	POE (10) [b] alphanol 79	15.9 [c]	0.5	0.78
–	POE (20) alphanol 79	17.7 [c]	0.9	1.27

[a] POE: polyoxyethylene.

[b] Alphanol: C_7–C_9 alcohol fraction.

[c] HLB calculated from molecular weights using HLB = 20×. 1 − Mol. wt. hydrophobic gp./Total mol. wt.

Single fish were placed in 200 ml of drug solution at room temperature, contained in 250 ml glass beakers. Overturn time was taken as the point when the fish appeared unable to maintain balance. All determinations of overturn time were made by one individual. Three types of experiments were performed: (1) the overturn time was assessed as a function of barbiturate concentration: (2) the overturn time at a selected barbiturate concentration was determined as a function of surfactant concentration; and (3) reversibility of surfactant effects was determined by immersing the fish in surfactant solution for 10 min, washing the fish in 4 changes of distilled water and exposing the fish to known concentrations of barbiturates. Overturn time was then assessed.

Not less than 5 fish were used for any one determination. In all the experiments described above, control groups of fish were used frequently to minimize the effects of variation between different consignments of goldfish. This variation, although slight, has been shown to occur previously (Levy and Anello, 1968; Nightingale et al., 1969).

Results

The overturn time (T_0) of goldfish varied inversely with the concentration of secobarbitone and thiopentone in aqueous solution (Fig. 1) showing agreement with the Levy and Gucinski (1964) equation. Phenobarbitone, the least potent substance, however, did not give a linear relationship (Fig. 1c) and while no quantitative difference in behaviour was detected, the results on phenobarbitone are not discussed further in this paper.

The effects on the activity of secobarbitone (0.2%) and thiopentone (0.03%) of the range of surfactants at constant concentration (0.1%) are shown in histogram form, firstly in Figs. 2 and 3 in relation to surfactant polyoxyethylene and alkyl chain length and secondly in Fig. 4 in relation to surfactant HLB. The results are expressed using an enhancement factor which is the turnover time produced by the barbiturate in aqueous solution divided by that obtained in surfactant solutions. The surfactants with short hydrophilic chains had little or no effect on barbiturate absorption, and because of their low solubility (less than 0.1%) and the consequent difficulty in preparing solutions, further discussion of these short chain compounds will be brief. They caused no enhancement of absorption at low concentrations (down to 10^{-4}%, approximately 10^{-7} M) thus it can be assumed that the low HLB surfactants have no measurable effect on the permeability of the absorbing membrane at concentrations at which they are in solution.

The greatest effect on overturn time appears to occur with surfactants based on a lauryl hydrocarbon chain (e.g. Brij 30, Brij 36T). These surfactants, however, possess some intrinsic biological activity (Florence et al., 1978). Addition of thiopentone to 0.01% surfactant solution has virtually no effect on the overturn times produced by these surfactants alone, but at 0.1% levels of Brij 36T there appears to be a marked synergism of effect between thiopentone and surfactant. It is thus difficult to include these surfactants in a generalized discussion in which we are trying to deduce a

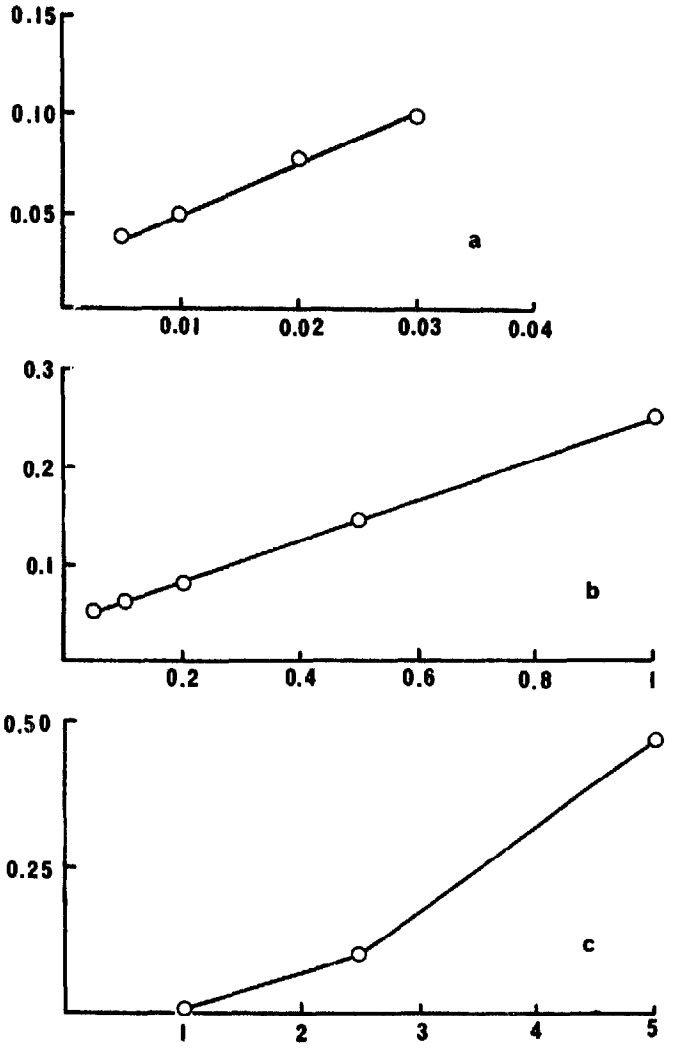

Fig. 1. Induced turnover times of goldfish as a function of drug concentration in the bathing solution for: (a) thiopentone; (b) secobarbitone; and (c) phenobarbitone. Ordinate: reciprocal turnover time (min^{-1}). Abscissa: drug concentration (% w/v).

physicochemical mechanism for increase in absorption. The other surfactants used in this work have no effect on the goldfish.

Exposure of the goldfish to 0.1% of Brij 76 and 78 for 10 min, followed by four 5-min washes in distilled water resulted in an enhancement of drug absorption when the fish were placed in 0.2% secobarbitone solution containing no surfactant (Table 2).

These results suggest that the surfactants can affect the absorption of secobarbitone in goldfish even when the two substances are not present together in the bulk phase of the solution. Pretreatment with Brij 72 had no effect on secobarbitone absorption, increasing the evidence that the very hydrophobic surfactants do not interact with biological membranes to alter their permeability characteristics.

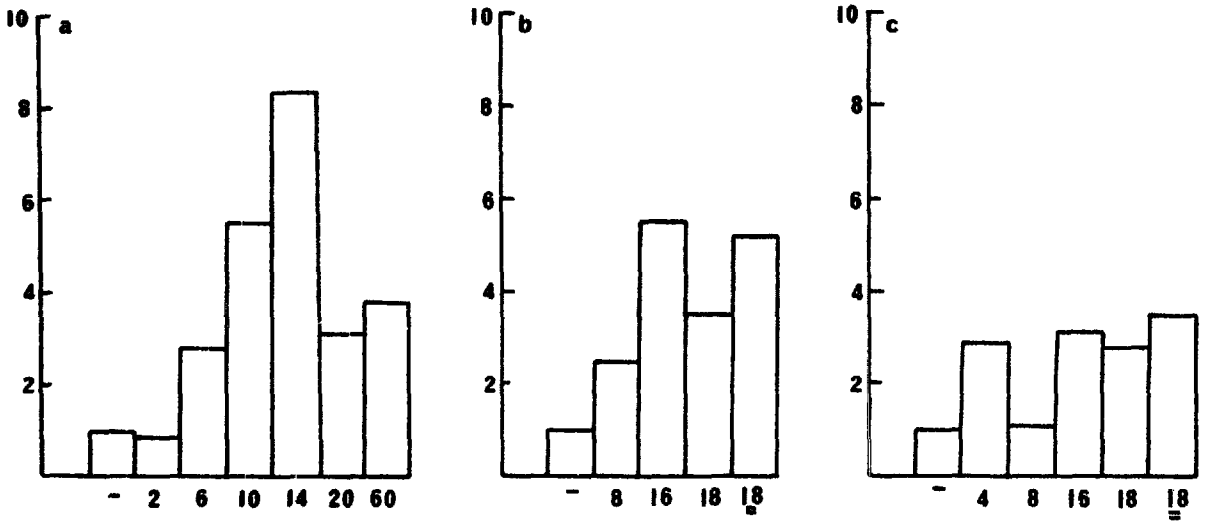

Fig. 2. Increases in the absorption of secobarbitone caused by several commercial non-ionic surfactants at 0.1% w/v levels. Ordinate in all cases the 'enhancement factor'. Abscissa: (a) polyoxyethylene chain length of surfactants based on C_{16} alkyl chain; (b) alkyl chain lengths of surfactants with 10 oxyethylene units; and (c) alkyl chain lengths of surfactants with 20 oxyethylene units.

TABLE 2

EFFECT OF PRETREATMENT WITH POLYOXYETHYLENE STEARYL ETHERS ON SECOBARBITONE ABSORPTION IN GOLDFISH

Surfactant	$1/T_0$ min^{-1} (for 0.2% secobarbitone) ± S.D.		
	Barbiturate alone	Barbiturate in 0.1% surfactant	Barbiturate alone after surfactant pretreatment
Brij 72	0.11 ± 0.02	0.09 ± 0.01	0.10 ± 0.01
Brij 76	0.11 ± 0.02	0.37 ± 0.04	0.28 ± 0.03
Brij 78	0.11 ± 0.02	0.31 ± 0.04	0.27 ± 0.04

Discussion

The relationship between reciprocal overturn time of the goldfish and secobarbitone and thiopentone concentration in the bathing medium was linear ($r = 0.990$ and 0.984, respectively), indicating agreement with the Levy-Gucinski equation. The deviation from linearity observed with phenobarbitone may be due either to the ability of the fish to metabolize low concentrations of this drug or to the relatively high concentrations of drug which had to be used. A linear relationship between phenobarbitone concentration and absorption has been demonstrated for gambusia fish (Khalil et al., 1976) a fish which appears to be more sensitive to barbiturates than the goldfish. A linear relationship between phenobarbitone absorption and

TABLE 3

RELATION BETWEEN BARBITURATE LIPOPHILICITY AND THE CONCENTRATION REQUIRED FOR A SPECIFIC EFFECT

Barbiturate	Concentration required for $1/T_0 = 0.1$ (% w/v)	o/w partition coefficient * (Bush, 1963)
Phenobarbitone	2.5	3
Secobarbitone	0.2	52
Thiopentone	0.03	580

* Methylene chloride/water.

pharmacological effect has previously been observed in goldfish (Levy and Miller, 1964) at low pH where activity is higher.

Presence of the surfactants in the bathing medium caused a concentration-dependent alteration in the absorption of thiopentone and secobarbitone. In the majority of cases detectable enhancement of absorption occurred at surfactant concentrations above 10^{-3}% (Tables 4 and 5).

The present work has employed the largest range of surfactants used in a study of this kind. The fact that no simple pattern of surfactant action on biological membranes has emerged points to the complexity of observed effects. Yet there is no doubt over the effectiveness of some of the non-ionic surfactants, the most active surfactant increasing absorption of secobarbitone by a factor of 8 times (Fig. 2a). One problem that emerges from consideration of the results in Figs. 2 and 3 is the different order of effects within a homologous series of surfactants, such as those with a C16 alkyl chain, shown clearly in Fig. 2a. Surfactants based on cetyl, stearyl and oleyl hydrocarbon chains with at least 10 ethylene oxide moieties all caused an

TABLE 4

RECIPROCAL TURNOVER TIMES FOR THIOPENTONE AS A FUNCTION OF SURFACTANT CONCENTRATION

Surfactant	Surfactant concentration (%)				
	0.001	0.005	0.01	0.05	0.1
C_{16} [a] EO_2 [b]	0.11 ± 0.04 [c]	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.02	0.08 ± 0.02
$C_{16}EO_{10}$	0.22 ± 0.04	0.31 ± 0.06	0.31 ± 0.06	0.5	0.5
$C_{16}EO_{20}$	0.29 ± 0.05	0.38 ± 0.13	0.38 ± 0.09	0.59 ± 0.09	0.61 ± 0.09
$C_{18}EO_2$	0.07 ± 0.02	0.20 ± 0.06	0.07 ± 0.01	0.06 ± 0.02	0.07 ± 0.03
$C_{18}EO_{10}$	0.15 ± 0.02	0.39 ± 0.03	0.39 ± 0.09	0.47 ± 0.07	0.41 ± 0.07
$C_{18}EO_{20}$	0.18 ± 0.01	0.33	0.32 ± 0.02	0.40 ± 0.09	0.31 ± 0.02
C_{18} [d] EO_2	0.10 ± 0.02	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.11 ± 0.01
$C_{18}^{=}EO_{10}$	0.19 ± 0.03	0.29 ± 0.04	0.31 ± 0.02	0.58 ± 0.13	0.34 ± 0.19
$C_{18}^{=}EO_{20}$	0.12 ± 0.03	0.27 ± 0.03	0.19 ± 0.02	0.29 ± 0.04	0.30 ± 0.02

For footnotes a–d, see Table 5.

TABLE 5

RECIPROCAL TURNOVER TIMES FOR SECOBARBITONE AS A FUNCTION OF SURFACTANT CONCENTRATION

Surfactant	Surfactant concentration (%)				
	0.001	0.0005	0.01	0.05	0.1
C_{16} [a] EO_2 [b]	0.11±0.02 [c]	0.11±0.01	0.09±0.01	0.09±0.01	0.09±0.01
$C_{16}EO_{10}$	0.38±0.09	0.37±0.04	0.44±0.05	0.70±0.20	0.60±0.08
$C_{16}EO_{20}$	0.17±0.02	0.19±0.02	0.20±0.02	0.33±0.08	0.35±0.06
$C_{18}EO_2$	0.11±0.01	0.10±0.01	0.10±0.01	0.09±0.01	0.09±0.01
$C_{18}EO_{10}$	0.21±0.01	0.19±0.01	0.19±0.02	0.27±0.03	0.37±0.04
$C_{18}EO_{20}$	0.16±0.02	0.23±0.03	0.25±0.02	0.33±0.08	0.31±0.04
C_{18} [d] EO_{10}	0.12±0.02	0.09±0.01	0.10±0.01	0.12±0.02	0.11±0.01
$C_{18}^{=}EO_{10}$	0.23±0.02	0.33±0.05	0.26±0.01	0.42±0.10	0.58±0.07
$C_{18}^{=}EO_{20}$	0.37±0.04	0.32±0.02	0.42±0.10	0.47±0.03	0.37±0.04

Secobarbitone concentration: 0.2%.

$1/T_0$ for secobarbitone in the absence of surfactant: 0.11±0.02 min^{-1}.

[a] C: alkyl chain length.

[b] EO: ethylene oxide chain length.

[c] Mean reciprocal turnover time±S.D. (n=5).

[d] Indicates unsaturated alkyl chain.

increase in the rate of barbiturate absorption. The greatest enhancement of absorption for each barbiturate occurred with surfactants with 10–20 ethylene oxide moieties. Neither surface activity, adsorption, area per molecule or critical micelle concentration shows a maximum which might explain such a maximum in biological activity. Neither does the hydrophile–lipophile balance (HLB) of the surfactant usefully predict activity (Fig. 4).

It seems likely that the polyoxyethylene alkyl ethers can interact with the gill membrane in two ways. Initially adsorption of the surfactant onto the surface of the membrane followed by penetration may occur at premicellar concentrations. Once

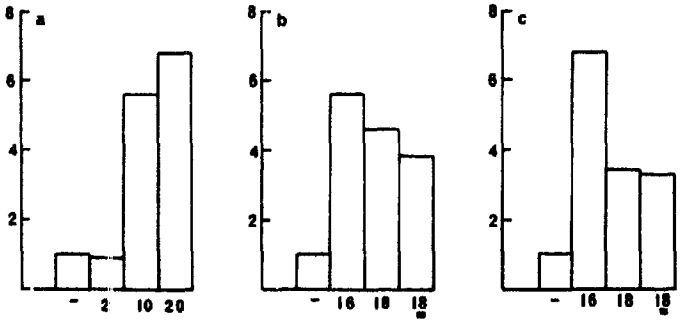

Fig. 3. Increases in the absorption of thiopentone caused by several commercial non-ionic surfactants at 0.1% w/v levels. Ordinate and abscissa as for Fig. 2.

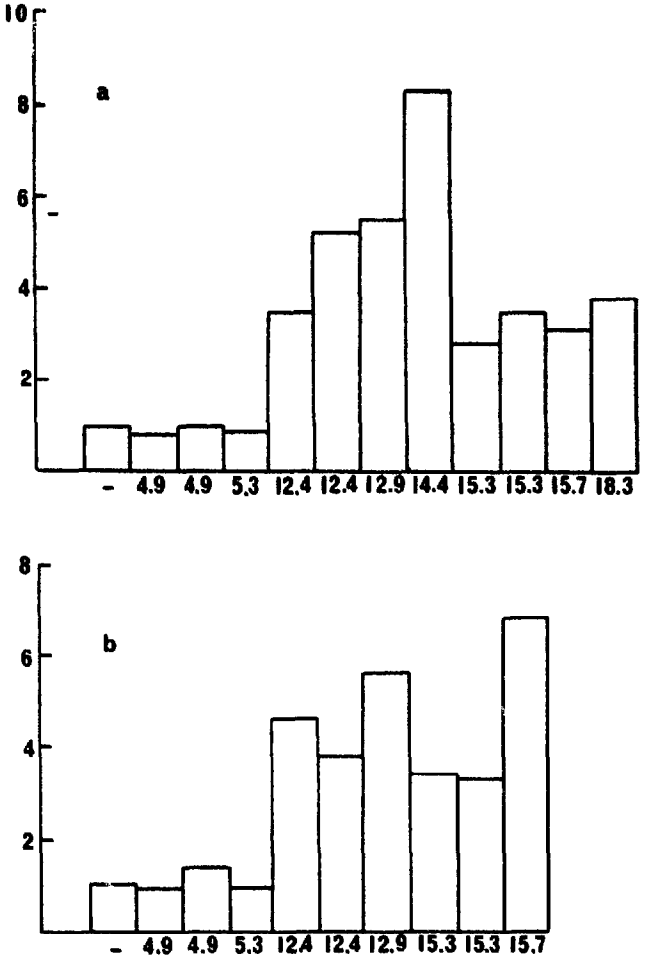

Fig. 4. Increases in the absorption of: (a) secobarbitone; and (b) thiopentone as a function of surfactant HLB. Ordinate: the 'enhancement factor'. Abscissa: surfactant HLB.1

inside the membrane the surfactant monomers are likely to increase fluidity of the hydrocarbon core (Levy et al., 1966). The increased fluidity may cause disorientation of membrane components and at micellar concentrations the surfactants may solubilize some of these components and cause disruption of the membranes. If penetration as well as adsorption is involved the cross-sectional area of the molecules should be one parameter that influences activity. The range of molecular areas obtained from surface tension measurements was not large enough for a categorical statement on the importance of dimensions. However, it appears that surfactants having C12–C16 hydrocarbon chains and polyoxyethylene chain lengths between 10 and 20, and molecular areas of between 1.00 and 1.60 nm^2 demonstrate the greatest enhancement of absorption. The physical bulk of the compounds with long ethylene oxide chains may explain the form of the results in Figs. 2 and 3. The more hydrophilic surfactants also have a lesser tendency to adsorb (Florence and Gillan, 1975). Insolubility of the surfactants with two ethylene oxide chains might explain

their lack of activity but the optimal effect of, for example, polyoxethylene (14) cetyl ether (Texofor A14) on secobarbitone absorption remains to be explained. Sufficient results have been obtained, however, to indicate that the alkyl chain length of the surfactant is of importance in the enhancement of membrane transport (Figs. 2 and 3).

It can be considered that the overall effect of the surfactants is the result of two opposing effects—interaction with the membrane and that of the permeant with the micelles. Preliminary experiments to determine interaction between the surfactants and the barbiturates used here have indicated that some solubilization of the barbiturates does occur but it is concluded that membrane surfactant interaction is the dominant effect in the system described here.

The surfactants used in this work were the same as those used in the study of gastric mucosal transport of paraquat (Walters et al., 1981). Despite the difference in the test penetrants and membrane systems, similar effects on transport properties were observed. The short ethylene oxide chain lengths and surfactants showed no transport enhancement activity in either system. An optimal effect was noted in both studies to be caused by surfactants with ethylene oxide chains of 10–20 units and alkyl chain lengths of C_{12}–C_{16}.

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