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Non-ionic surfactants and gastric mucosal transport of paraquat[1]

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The influence of a range of polyoxyethylated non-ionic surfactants upon the transport of [^{14}C]-paraquat dichloride across rabbit isolated gastric mucosa was investigated. Paraquat was shown to cross the mucosal epithelium by passive diffusion. Certain members of the surfactant series enhanced transmucosal paraquat transfer at low surfactant concentration (e.g. 0·001 %) but the occurrence and magnitude of this effect was not dependent in a simple manner upon surfactant structure or physicochemical properties. At micellar concentrations the increase in paraquat transport was greatest with those surfactants possessing both ethylene oxide chains of 10–20 units and alkyl chains longer than C_7–C_9. The most effective absorption promoter was found to be Brij 36T ($C_{12}E_{10}$). At micellar surfactant concentrations, the enhancement of paraquat transfer appeared, from histological evidence, to be related to the ability of the surfactants to solubilize membrane components and disrupt epithelial cells.

Surfactants are present in many pharmaceutical formulations and in foodstuffs and are known to affect the absorption of substances from the gastrointestinal tract (Gibaldi & Feldman 1970). Little is known of the mechanisms underlying surfactant effects on biological membranes such as those lining the epithelium of the gastrointestinal mucosa.

Many reports have appeared on the effects of surfactants in biological systems and several authors have attempted to relate these effects to the physicochemical properties of the surfactant and have met with varying degrees of success (Egan et al 1976; Slinde & Flatmark 1976; Marsh & Maurice 1971; Smith et al 1966; Zaslovsky et al 1978). The common theme of many publications on the biological effects of surfactants is the existence of a concentration-dependent biphasic action (Florence & Gillan 1975a), that is to say an enhancement of transport at low concentrations of surfactant but a decrease at higher concentrations, generally above the critical micellar concentration of the surfactant (Florence & Gillan 1975b). Reduction of transport of a drug in surfactant systems is attributed to the ability of the surfactant to form micelles and is normally only observed if interaction between micelle and drug occurs. Increase in membrane transport at low surfactant concentrations is normally attributed to the amphipathic nature of the surfactant and its ability to penetrate, and thus disrupt, normal membrane structure. Release of membrane proteins has been found to be accompanied by large increases in absorption rates of both saline and salicylate through rat jejunal tissue (Whitmore et al 1979). The results presented here form part of an investigation of the effects of non-ionic surfactants on gastrointestinal absorption. The use of in vitro methods allows the exposure of defined regions of epithelium to known concentrations of absorbant under controlled conditions. The effects of a series of polyoxyethylene alkyl surfactants on the isolated gastric mucosa of rabbit were measured using radiolabelled paraquat dichloride as test absorbant. The passive character of paraquat diffusion across the mucosal preparation was established and the lack of any interactions between the paraquat ion and surfactant molecules or micelles in solution was confirmed. Through selection of surfactants with graded lipophile and hydrophile chain lengths, an examination was possible of the influence of surfactant structure and physicochemical properties on paraquat transport.

MATERIALS AND METHODS

Animals. Female New Zealand white rabbits (2–3 kg) obtained from Hacking and Churchill Limited, Huntingdon, England, were used.

Surfactants. Polyoxyethylene alkyl ethers of the Brij series were obtained from ICI United States Inc., Wilmington, Delaware, except for polyoxyethylene

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(10) lauryl ether (Brij 36T) which was obtained from Sigma London Chemical Company Limited, Poole, Dorset.

Polyoxyethylene (40) stearate (Myrj 52) was obtained from ICI United States Inc. Surfactants of the Texofor series were obtained from ABM Chemicals, Stockport, Cheshire. Short alkyl chain polyethoxylated alcohols, polyoxyethylene (20) butanol, polyoxyethylene (10) alphanol 79, and polyoxyethylene (20) alphanol 79 were obtained from ICI Organics Division, Blackley, Manchester. Hydrophile-lipophile balance (HLB) values quoted in this paper are those provided by the manufacturers.

Marker compound: Paraquat (1,1′-dimethyl-4,4′-bipyridylium) dichloride was obtained from ICI Plant Production Limited, Bracknell, Berkshire and [^{14}C]methyl paraquat (specific activity 30 mCi m mol^{-1}) was obtained from the Radiochemical Centre, Amersham.

All reagents were used as supplied. Aqueous solutions were made using distilled water.

Determination of the physicochemical properties of surfactant solutions. Effects of surfactants on the surface tension of water. Surface tensions (σ) were measured at room temperature using a glass Wilhelmy plate (4·4 cm perimeter) and a torsion balance (0–500 mg). Graphs of surface tension against log surfactant concentration (c) were drawn, and the simple form of the Gibbs' equation,

$$\Gamma = -\frac{1}{2{\cdot}303\ RT}\frac{d\sigma}{d\log c}$$

was used to calculate the surface excess concentration (Γ). The area per molecule (A nm^2) at the air-water interface was then calcuated from the relation

$$A = \frac{10^{18}}{\Gamma N}$$

where N is Avogadro's Number.

Interaction between surfactants and the marker compound. Interaction between paraquat and the non-ionic surfactants was determined by a non-equilibrium dialysis technique at room temperature (20 °C). Two 5 ml capacity glass half-cells were separated by a Visking cellophane membrane. This type of cellulose membrane is generally considered to be permeable to small molecules but not to the surfactant micelles (Barry & El Eini 1976b). Before use, the Visking tubing was boiled in distilled water for several minutes and allowed to cool. Radiolabelled paraquat buffered (composition of physiological buffer solution used mM: sodium chloride, 107; potassium chloride, 5; sodium bicarbonate, 25; calcium chloride, 1; magnesium chloride, 1; potassium dihydrogen phosphate 1; glucose, 20) in surfactant solutions of various concentrations was placed in one cell, buffer alone in the other and the rate of transfer of radiolabel into the recipient cell determined over 2 h. In all cases the increase in concentration with time was linear and the rate of dialysis was proportional to the concentration of paraquat.

Absorption experiments

Rabbits were killed with an overdose of pentobarbitone (Nembutal), administered intravenously. The stomach was rapidly removed, opened along the lesser curvature and the fundus and antrum separated. The fundus was washed with sterile 0·9% sodium chloride and placed on a wax tray containing buffer solution with the mucosal side down. The buffer solution in the wax tray was gassed with 5% carbon dioxide in oxygen to maintain the tissue as viable as possible during the stripping procedure.

The external muscle coats were removed by blunt dissection, to minimize the possibility of perforating the epithelial layer, and a preparation consisting of mucosa and muscularis mucosa was obtained. This preparation was then set up between two halves of a diffusion chamber described below. Six samples of mucosa from each rabbit and six diffusion chambers were used for each experiment. The elapsed time between excision and bathing with oxygenated buffer at 37 °C in the diffusion chamber rarely exceeded 15 min. All manipulations outside the diffusion chamber were at room temperature.

The diffusion chamber

The Perspex diffusion chamber was based on that used by Ussing & Zehran (1951) and allowed the exposure of 1·77 cm^2 of the mucosal and serosal surface of the tissue to oxygenated physiological buffer solution (pH 7·0). The comparatively low length of edge to surface area ratio for the tissue when set up in the chamber indicates that no significant edge damage will have occurred (Helman & Miller 1971). The buffer solution was maintained at 37 °C by means of a water jacket connected to a constant temperature circulating pump. The bathing medium was stirred and oxygenated by a gas-lift mechanism, utilizing 5% carbon dioxide in oxygen, humidified by bubbling through water and heated by

passing through a water bath at 37 °C. The chambers were plugged with a rubber bung through which passed a thistle funnel to act as a condenser. These refinements minimized evaporative loss of the bathing medium and the decrease in the volume of the perfusate was limited to 5 to 7 per cent over 3 h. Results obtained from chambers whose volume decrease was greater than 7 per cent were discarded. Tissue could be maintained in the diffusion chambers for at least 3 h without loss of viability or integrity as judged by histological assessment and by transmucosal potential difference measurements (Corbett et al 1977).

Determination of the mode of transport of paraquat

To determine the effects of paraquat concentration on the rate of paraquat transfer, isolated gastric segments were set up in the diffusion chambers and the mucosal surface was exposed to paraquat dichloride solutions ranging in concentration from 1–100 mg ml^{-1}. [^{14}C]Methyl paraquat (10 μCi) was added to the mucosal, or donor, solution. Samples (50 μl) were taken from the donor solution at the beginning and end of each experiment. Samples (50 μl) were taken from the receptor solution at intervals up to a maximum of 3 h. Radioactivity in the samples was determined using an Intertechnique SL30 scintillation counter and a toluene-based scintillation fluid (Permafluor 1, Packard) containing 375 ml methanol in 2·5 litres. The quenching factor for samples was shown to be constant and thus no correction was required. To determine any directional preference to paraquat transfer which could indicate active transport or carrier mechanism, both mucosal to serosal and serosal to mucosal fluxes were estimated. Transfer of paraquat was also determined when the concentration of the absorbant was similar on both sides of the membranes.

The effect of the metabolic inhibitors 2,4-dinitrophenol (1 and 10 mM) and sodium cyanide (5 and 50 mM) on the rate of paraquat transfer was determined.

Effects of surfactants on paraquat transfer

The mucosal surface was exposed to paraquat solution (1 mg ml^{-1}) in the presence of varying concentrations of surfactants (0·0001–1·0%) and transfer of paraquat was determined as described above. To minimize osmotic effects, equal amounts of surfactants were placed on both sides of the tissue. Transfer of paraquat was determined over a 3 h period.

With several of the surfactants, recovery experiments were performed by washing the tissue, after exposure, with four changes of buffer solution. Transfer of paraquat in the absence of surfactant was then determined.

Samples of gastric mucosa which had been placed in the diffusion chamber in the presence and absence of surfactants were fixed in a mercuric chloride fixative. The samples were processed, stained with haematoxylin and eosin and examined to ascertain morphological changes in the mucosa.

Samples of the mucosal bathing fluid were taken at the end of several experiments to determine the level of protein extraction. Protein was estimated quantitatively by the method of Lowry et al (1951) and results are quoted in bovine serum albumin equivalents. Surfactants were placed in the appropriate concentration in the standard solutions to obviate the problems of interference.

RESULTS AND DISCUSSION

Physical properties of the surfactants

The critical micelle concentrations and limiting surface tensions of the surfactant obtained from plots of surface tension vs log (concentration) are listed in Table 1. The dialysis experiments indicated that no significant interaction occurred between paraquat and surfactants.

Mode of transport of paraquat across isolated gastric mucosa

Although the functional integrity of everted intestinal sacs over a period of time has been questioned (Levine et al 1970; Gibaldi & Grundhofer 1972), the type of 'stripped' mucosal preparation we used can maintain a viable state for several hours (Field et al 1971; Corbett et al 1977). Thus any active processes involved in paraquat transfer across the epithelium would remain operative. The results of the investigation of the mechanism of paraquat transfer show, however, that processes requiring metabolic energy are not involved and that absorption probably occurs by simple passive diffusion.

Accumulated activity in the receptor solutions of the diffusion chamber increased linearly with time in all cases. Permeability constants were calculated from the slope of the plot of activity versus time by application of Fick's law of diffusion. Thus the slope of the line, corrected for tissue area yields J, the flux (absorption rate per unit area) which, divided by the concentration of solute applied, (c) gives the permeability constant, K_p. Table 2 shows the permeability constants of 10 μCi[^{14}C]methyl paraquat across rabbit gastric mucosa from varying concentrations of unlabelled paraquat.

Table 1. Surface properties of polyoxyethylene non-ionic surfactants.

Surfactant	Type	HLB	Cmc (%w/v)	Area/mol (nm^2)	Limiting surface tension ($m\ Nm^{-1}$)
Brij 30	POE*(4) lauryl ether	9·7	10 0·006	0·38	27·5
Brij 35	POE (23) lauryl ether	16·9	0·01	1·15	38·0
Brij 36T	POE (10) lauryl ether	15·2‡	0·008	1·09	29·5
Brij 52	POE (2) cetyl ether	5·3	0·003	0·27	31·4
Brij 56	POE (10) cetyl ether	12·9	0·006	1·10	30·4
Brij 58	POE (20) cetyl ether	15·7	0·01	1·48	34·6
Brij 72	POE (2) stearyl ether	4·9	0·002	0·30	35·2
Brij 76	POE (10) stearyl ether	12·4	0·005	1·06	30·6
Brij 78	POE (20) stearyl ether	15·3	0·009	1·54	40·0
Brij 92	POE (2) deyl ether	4·9	0·002	0·29	34·0
Brij 96	POE (10) oleyl ether	12·4	0·006	1·03	29·5
Brij 98	POE (20) oleyl ether	15·3	0·01	1·51	33·9
Myrj 52	POE (40) stearate	16·9	0·03	2·34	44·0
Texofor A6	POE (6) cetyl ether	5·3	0·002	0·60	42·4
Texofor A14	POE (14) cetyl ether	14·4	0·007	1·20	31·2
Texofor A60	POE (60) cetyl ether	18·3	0·06	2·56	41·0
—	POE (20) butanol	18·8‡	—	1·15	—
—	POE (10)† alphanol 79	15·9‡	0·5	0·78	31·8
—	POE (20) alphanol 79	17·7‡	0·9	1·27	39·2

* POE: polyoxyethylene.
† alphnol: C_7–C_9 alcohol fraction.
‡ HLB calculated from molecular weights using HLB = 20 x. $\left[1 - \frac{\text{Mol. wt Hydrophobic gp.}}{\text{Total Mol. wt}}\right]$

The rate of transfer is proportional to concentration up to 10 mg ml^{-1}, and the permeability constant is independent of concentration, thus diffusion may be considered to be Fickian. From 20–100 mg ml^{-1}, however, the rate of transfer increases dramatically. At the higher concentrations paraquat may exert a toxic action on the mucosa, probably resulting in mucosal disruption.

Table 2. The effect of concentration of paraquat on transfer rate.

Paraquat concn (ion) in donor side (mg ml^{-1})	Paraquat permeability constant ($cm\ h^{-1} \times 10^3$) (with s.d.)
1	10·5 (3·9)
5	9·7 (2·9)
10	10·5 (2·8)
20	22·3 (7·2)
50	38·7 (9·8)
100	30·8 (8·4)

No. of experiments = 6.

Transfer of paraquat from the serosal surface to the mucosal surface occurs at a similar rate to mucosal to serosal transfer ($8·98 \pm 3·6 \times 10^3$ cm h^{-1} and $10·5 \pm 3·9 \times 10^3$ cm h^{-1} respectively) indicating that there is no significant directional preference. Net transfer of paraquat did not occur against a concentration gradient, nor did it occur when concentrations of paraquat on both sides of the membrane were similar. Addition of the metabolic inhibitors 2,4-dinitrophenol (DNP) and sodium cyanide did not decrease the rate of paraquat transfer and therefore metabolic energy is not required for paraquat absorption across the mucosal preparation. Typical results for the permeability constant of paraquat in the presence of inhibitors were $11·8 \pm 1·0 \times 10^{-3}$ cm h^{-1} (10 mM DNP) and $10·6 \pm 1·8$ (50 mM NaCN).

These data indicate that no active or facilitated processes occur during paraquat absorption by the isolated fundic mucosa of the rabbit. It seems likely, therefore, that absorption occurs by simple passive diffusion.

The effect of surfactants on paraquat transfer

Permeability constants of paraquat were calculated as above. The effects on paraquat transfer of all the surfactants studied are summarized in Table 3. The overall mean permeability constant (150 observations) of paraquat through the gastric mucosa of rabbit was $9·0 \pm 4·0 \times 10^{-3}$ cm h^{-1} from a 1 mg ml^{-1} paraquat ion donor solution. In the presence of surfactants of varying lipophilic and hydrophilic character, the permeability constant of paraquat was altered, depending on the concentration of surfactant. Even at 0·001% several surfactants caused a significant increase in the rate of paraquat transfer but the increase in permeability at all concentrations of surfactant does not appear to be simply related to any of the physicochemical properties of the surfactants such as HLB or ethylene oxide chain length, in agreement with results obtained with other systems such as goldfish (Florence & Gillan 1975a, b) or observations on surfactant effects on corneal permeability (Marsh & Maurice 1971). However, if the present results are considered by collating results on surfactants either with a constant alkyl chain length or a constant polyoxyethylene chain length some patterns of behaviour do emerge. In Fig. 1 results are plotted for the C_{16} compounds as a function of ethylene oxide chain length from n = 2 to n = 60. Of the results at low surfactant concentration (Fig. 1a) only three surfactants produce paraquat transport which is significantly different from that of the

Table 3. Concentration dependent effects of surfactants on paraquat transfer across isolated gastric mucosa.

Concn (%) of surfactant	Paraquat permeability constant (cm $h^{-1} \times 10^3$) (with s.d.) 0·0001	0·001	0·01	0·1	1·0
No surfactant		9·0 ± 4·0			
Brij 30	24 (5)	11 (7)	9 (2)	17 (1)†	7 (2)
Brij 35	13 (6)	14 (4)*	14 (5)	22 (9)*	61 (32)*
Brij 36T	11 (5)	11 (6)	25 (16)	50 (39)	146 (20)†
Brij 52	—	13 (4)	—	—	8 (2)
Brij 56	—	18 (6)*	—	—	52 (11)†
Brij 58	—	11 (1)*	—	—	36 (13)***
Brij 72	31 (7)***	15 (4)*	9 (2)	9 (3)	8 (4)
Brij 76	12 (3)	13 (4)	16 (5)*	45 (21)**	42 (14)***
Brij 78	15 (4)*	22 (5)***	30 (11)**	43 (23)*	25 (7)***
Brij 92	5 (2)**	27 (12)*	6 (3)	6 (1)***	6 (3)
Brij 96	9 (4)	13 (8)	19 (6)*	27 (6)***	83 (31)***
Brij 98	7 (2)	17 (9)	12 (6)	71 (6)†	68 (28)*
Myrj 52	12 (4)	7 (3)	6 (2)*	9 (3)	7 (3)
Texofor A6	—	8 (2)	—	—	10 (3)
Texofor A14	—	22 (7)**	—	—	39 (7)†
Texofor A60	—	12 (4)	—	—	28 (11)**
POE (20) butanol	—	6 (2)*	—	—	5 (2)**
POE (10) alphanol 79	—	6 (1)***	—	—	9 (4)
POE (20) alphanol 79	—	15 (3)**	—	—	12 (3)

Levels of significance: * $P<0·05$
** $P<0·02$
*** $P<0·01$
† $P<0·001$
At least 5 determinations at each concentration.

control. Maximum effect is produced by $C_{16}E_{10}$ and $C_{16}E_{14}$. At 1% surfactant level it is seen that $C_{16}E_{10}$ has the largest effect on transport, the value of K_p falling with increasing hydrophilic chain length. The effect of alkyl chain may be adduced from the results plotted in Fig. 2. In the series of surfactants with 10 ethylene oxide units and saturated alkyl chains the C_{12} compound has the greatest effect on paraquat permeability. As is expected from the general trend with hydrophilic chain length the more hydrophilic compounds in Fig. 2b are less effective promoters of absorption. However the lauryl derivative has the

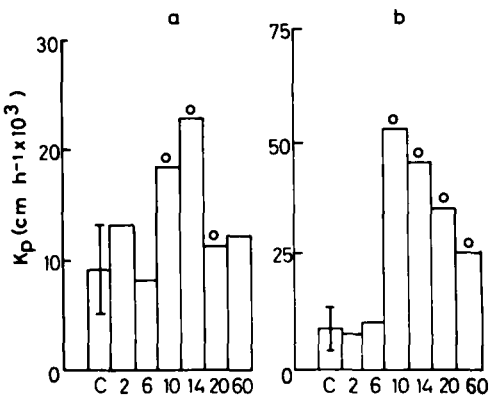

FIG. 1. Values of K_p for paraquat obtained a) at 0·01% surfactant levels and (b) at 1·0% surfactant levels using isolated rabbit gastric mucosa as a function of ethylene oxide chain length. The surfactants have a C_{16} alkyl chain. Ethylene oxide chain lengths are marked on the abscissa from E_2 to E_{60}. C is the control, paraquat without additives. Results which are statistically significantly different ($P<0·05$) from the control values are marked (○).

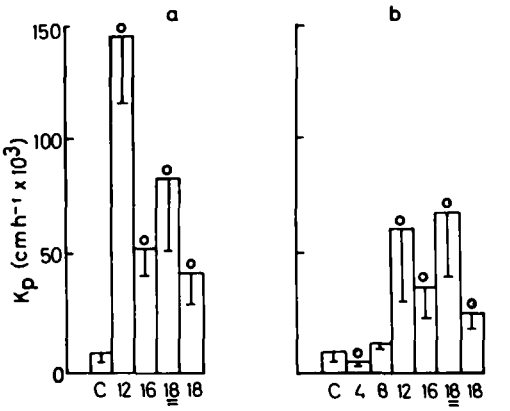

FIG. 2. Values of K_p for paraquat obtained at 1·0% surfactant levels with isolated rabbit gastric mucosa as a function of alkyl chain: C_{12}, C_{16}, C_{18} and oleyl, marked 18 on the abscissa. (a) compounds with 10 ethylene oxide units and (b) compounds with 20 ethylene oxide units. Results which are statistically significantly different ($P < 0·05$) from the control values without surfactant are marked (⌒).

most pronounced effect. The unsaturated oleyl derivatives are more effective in increasing absorption than their saturated analogues. Fig. 2b has results for alkyl chain lengths for C_4 to C_{18} and demonstrates clearly the influence of the hydrocarbon chain. Each series either homologous in alkyl chain or ethylene oxide chain length displays a maximum leading to the conclusion that some optimal activity is required for maximal effect on membrane permeability. But the reason for a lack of obvious correlation when K_p is plotted against surfactant hydrophile-lipophile balance must be that the size and shape of the molecule are important. Surfactant molecules of the alkyl polyoxyethylene ether class of quite different molecular size e.g. $C_6H_{13}[OCH_2CH_2]_6OH$ and $C_{12}H_{25}[OCH_2CH_2]_{12}OH$, can have identical HLB values.

Marsh & Maurice (1971) found that while Brij 35 ($C_{12}E_{23}$) caused a marked rise in the penetration of fluorescein into the eye, Myrj 52 although having the same HLB (16·9) appeared to have little influence on corneal permeability. Brij 58 ($C_{16}E_{20}$) also caused a considerable rise in permeability but produced 'alarming' epithelial changes. Our results (Table 3) confirm these findings. At 1% levels K_p with Brij 35 is $61 \pm 32 \times 10^{-3}$ cm h^{-1} and in the presence of Myrj 52 it is $7 \pm 3 \times 10^{-3}$ cm h^{-1}. Damage to the membrane can result from extraction of components vital for the maintenance of membrane integrity such as cholesterol and phospholipids. The high and erratic value of K_p caused by high levels of some of the surfactants (Table 3) probably indicate a loss of membrane integrity. In order to discern the influence of structure on one such process—protein extraction—the effect of Brij 72, 76 and 78 on protein extraction from the membrane under study was investigated (Fig. 3). Measurable increases in protein extraction only occurred above the critical micelle concentration of the surfactant.

1% polyoxyethylene (10) stearyl ether (Brij 76) and polyoxyethylene (20) stearyl ether (Brij 78) had a dramatic effect on the extraction of protein from the gastric mucosa during absorption experiments. Polyoxyethylene (2) stearyl ether (Brij 72) has a very small effect on protein extraction and on K_p. Brij 76 and 78 which extract four times as much protein as Brij 72 both producing values of $K_p > 40 \times 10^{-3}$cm h^{-1}.

Histological investigation showed that all surfactants which increased the rate of paraquat absorption at higher concentrations also caused disruption of the mucosal border. Superficial epithelial cells were damaged by surfactant whilst those lying more

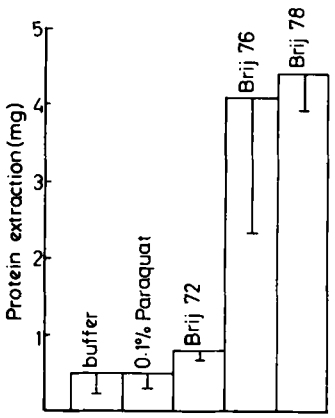

FIG. 3. Protein extraction (mg) in bovine serum albumin equivalents occurring in 3 h following incubation in the medium as marked. Surfactant concentration 0·1%.

deeply in the gastric pit often appeared unaffected. Little or no damage was evident after contact with those surfactants found to have no effect on paraquat transfer. Return to normal transfer rates did not occur after exposure of tissue to damaging surfactant solutions. It has been previously suggested (Florence & Gillan 1975a) that the area per molecule of the surfactant at the membrane interface, its cmc and its ability to solubilize drug molecules are parameters that determine a surfactant's effect on drug membrane transport. It is generally assumed that the cross-sectional area of the hydrophilic component determines the area per molecule of surfactants at aqueous solution interfaces (Schick 1962; Barry & El Eini 1976a). The measured molecular areas at the air-water interface are an approximation of the experimentally inaccessible membrane-water interfacial

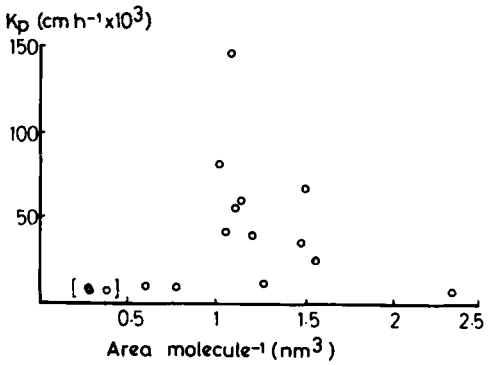

FIG. 4. The relationship between values of K_p for paraquat obtained on addition of surfactant to the system at the 1% level as a function of area/molecule (nm^3) of the surfactant at the air/water interface.

area but, nevertheless, a large cross-sectional area would represent one factor preventing ready penetration of membrane lipids by surfactant molecules.

Surfactants which enhance paraquat transport at 1·0% levels possessed areas per molecule greater than 1·0 nm^2 (Fig. 4) but the effect decreases with increasing size which is in general agreement with the observations of Florence & Gillan (1975b).

The study forms part of a programme aimed at a general understanding of surfactant effects on passive diffusion across epithelial membranes, whether the absorbant is a toxic chemical or a drug. Paraquat has been used here as a convenient divalent test absorbant that diffuses passively across the gastric mucosa. It is not possible to interpret the results obtained in terms of the toxicology of commercial paraquat formulations because of the dissimilarity of surfactants, complexity of formulations and the absence, in vitro, of physiological factors important in absorption.

Of the surfactants studied the most active was found to be Brij 36T ($C_{12}E_{10}$). The C_{12} derivatives of several surfactant series have been found to be more potent than higher or lower members in their adverse effects on skin (Ferguson & Prottey 1976) and as antimicrobial agents (Kabara 1978).

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