Surfactant depolarization of frog skin

The ability of surface-active agents to depolarize nerve (Walsh & Lee, 1963), muscles (Wasano & Goto, 1956) and frog skin (Schoffeneils, Gilles & Dandrifosse, 1962; Webb, 1965) has been previously demonstrated. However, a relation between the physical-chemical properties of the surfactants and their action on these systems has not so far been established. This contrasts with work on the lysis of single cells, for example, which showed a maximum in surfactant lytic power at the surfactant critical micelle concentration (cmc) (Pethica & Schulman, 1953).

We have investigated the depolarization of skins from two species of frogs, *Rana temporaria* and *R. pipiens*, by using increasing concentrations of surfactant. The nature of the surfactant-epithelium interaction was also examined using the microelectrode technique.

Ventral skins were mounted in a Ussing-type double chamber apparatus (Ussing, 1949) and bathed on both sides with either chloride or sulphate Ringer medium. The depolarization was observed on adding detergent to the outside rather than the inside surface since the effect is then greater. During the initial experimentation, it was found that the skin potential and its depolarization rate were not only a function of the detergent concentration, but also of the hydrostatic pressure across the skin. In general, a positive pressure in the outside compartment decreased the skin potential (Voote & Ussing, 1970) and increased the depolarization rate, while the opposite effect was obtained with a negative pressure. Therefore, hydrostatic effects accompanying solution addition were minimized. The skin potential difference (p.d.) was measured using a high impedance voltmeter or chart recorder connected to the cell via calomel electrodes and agar bridges. Resistance was measured by passing a current of 10–50 μA cm^{-2} through silver/silver chloride electrodes in the cell and measuring the change in p.d. Microelectrode measurements were made with the skin lying horizontally, outside uppermost, on a fine steel mesh with aerated medium circulating below. Microelectrodes filled with 3M KCl (Wann & Goldsmith, 1972) penetrated the skin perpendicularly from the outside. Electrodes in the upper and lower halves of the cell allowed the p.d.'s and impedances of the skin and microelectrode to be monitored during these experiments. Medium aeration was stopped to minimize distortion of the skin during impalement. Having established the two-step p.d. profile (Cereijido & Curran, 1956) which could be measured using this technique, an electrode was advanced into the skin until it recorded the first step and gave a steady p.d. before the test solutions were added to the skin.

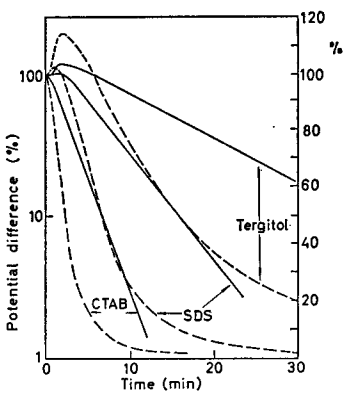

FIG. 1. A typical result of depolarization of *R. temporaria* skin by 5 × 10^{-4}M CTAB, SDS and Tergitol 15–S–9 (– – –); with data replotted logarithmically to show the kinetics are first order with respect to time (——).

The cmc of each surfactant in the appropriate medium was obtained from the surface tension concentration relation using either the du Nouy ring or Wilhelmy plate technique (Adamson, 1967) (measurements were reproducible to better than $\pm$ 0·5 dyne cm^{-1}). Values so obtained were in agreement with previous measurements (Matijevic & Pethica, 1958).

Experiments were conducted at room temperature. The two ionic surfactants, sodium dodecyl sulphate (SDS) and cetyl trimethylammonium bromide (CTAB) were ex BDH, and the non-ionic, Tergitol 15-S-9, was ex Union Carbide. The latter was included only to determine the validity of a previous statement (Schoffeneils & others, 1962) that a non-ionic surfactant had little depolarizing effect. Since, in general, the action of surfactants is influenced by their ionic environment, as is also the p.d. of the skin by its bathing medium, effects in two quite different media are reported. The experiments on *Rana temporaria* were conducted using a chloride, calcium/magnesium-free Ringer having the composition (mM) of: NaCl 111, KCl 2·0 and $NaHCO_3$ 2·4, adjusted to pH 7·5 and the experiments on *Rana pipiens* were conducted using sulphate* Ringer prepared by equimolar replacement of Cl^- by the less permeable SO_4^{2-} and containing 1 mM $CaSO_4$ in addition.

The time-course of depolarization is shown in Fig. 1. Potential difference is expressed as a percentage of the initial p.d. before insult. An initial increase in p.d. was often recorded after which it generally fell following kinetics first-order with respect to time, and allowed the determination of a rate constant, K (min^{-1}). (In some experiments, particularly those employing very low concentrations, zero order kinetics were observed). From Fig. 1 it is seen that a similar kinetics pattern was obtained from anionic, cationic and non-ionic detergents. Concentration effects were studied for the former two in detail. As the concentration of detergent increased, the rate of depolarization increased and a rapid increase in K occurred on passing the cmc (Fig. 2). This was less distinct with *R. temporaria* which showed more scatter in the data. This scatter is partly due to the difficulty in measuring the much higher K values recorded and partly to skin sloughing (Larsen, 1970) which could make the skins more sensitive to the action of the surfactant, and so enhance existing species differences.

Phenomenologically, the polarization potential can be described as being directly proportional to the rate of an unspecified metabolic reaction. The rate of decay

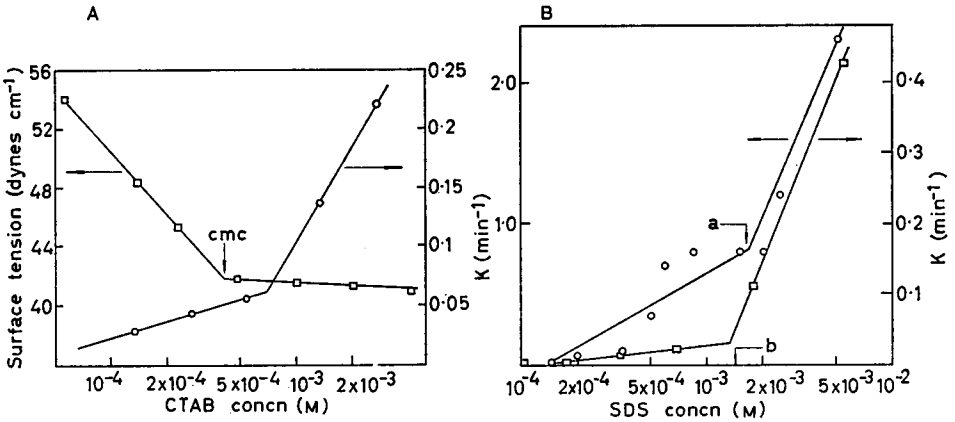

FIG. 2A. Effect of CTAB concentration on *R. pipiens* depolarization rate constant, K, and determination of cmc from surface tension. Each point is the average of 3–4 experiments (s.d. < 10%).

B. Effect of SDS concentration on depolarization of *R. temporaria* (○) and *R. pipiens* (□). Average of 3–4 experiments (s.d. for *R. temporaria* 20–60% and for *R. pipiens* <10%). a, cmc in chloride-Ringer; b, cmc in sulphate-Ringer.

* Sulphate was chosen as an alternative to chloride because it gave higher skin p.d.s.

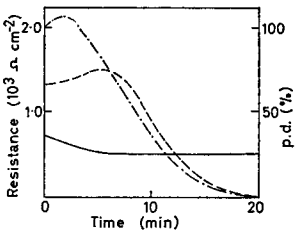

FIG. 3. A typical result of changes on total skin p.d. (–·–·–), inner resistance (——) and outer resistance (– – –) of *R. temporaria* when exposed to 7×10^{-4}M SDS.

would then be limited by the rate of diffusion of detergent in these reaction sites. An increase in the rate of depolarization above the cmc would, however, be surprising if the response were limited by the diffusion of material across the intact *stratum corneum* since this diffusion would be monomer-dependent. It must, therefore, be concluded that the corneal barrier is absorbing appreciable amounts of surfactant, and being degraded. Where this reaction involves lipid or protein extraction and solubilization the effect would increase above the cmc. An ultraviolet spectrophotometric analysis of the bathing medium after insult confirmed that some protein was being extracted. However, a light microscope histological examination of skin (haematoxylin and eosin stained cryo-sections) showed no significant change in its overall morphology. That a change does occur at the outer layers of the skin was confirmed by recording resistance changes during insult (Fig. 3). This is in agreement with previous observations that ionic permeability increases (Webb, 1965). Noting that the resistance of the inner region does not change appreciably even when the p.d. is zero, it may be concluded that the surfactants can inhibit the ion pump mechanism before gross changes in the membrane occur. This conclusion is further supported by observations that resistance may remain unchanged (or increase) during some surfactant treatments while the p.d. may be falling. These surfactant mediated changes are partly reversible since some recovery of the p.d. is seen on washing the skin with surfactant-free medium. However, the degree of recovery is very dependent on the concentration of surfactant used and the duration of exposure to the surfactant.

Although we have shown that the depolarization kinetics are similar for anionic, cationic and non-ionic surfactants, appreciable differences in rate constants are evident. Furthermore, although a similar order of effectiveness, namely CTAB> SDS>non-ionic may be observed when epithelia such as bovine cornea (Carter, Duncan & Rennie, 1973) is insulted, extrapolation to other epithelia and insult conditions must be cautious since depolarization rate constants vary even between frog species as shown above, and the physical chemistry of surfactants is influenced by the ionic environment.

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Effect of some anorectic agents on the uptake and release of 5-hydroxytryptamine by blood platelets of rats

Previously fenfluramine has been shown to differ from amphetamine at $2{\cdot}5 \times 10^{-5}$M in that it inhibits 5-hydroxytryptamine (5-HT) uptake and releases 5-HT from rat platelets (Buczko, de Gaetano & Garattini, 1975). Whether other anorectic agents share with fenfluramine its effects on rat platelet 5-HT has now been investigated.

Male Charles River rats (170–200 g) were used. Platelet-rich plasma (PRP) and platelet-poor plasma were prepared and ^{14}C-5-HT uptake and release measured according to Buczko, de Gaetano & Garattini (1974, 1975).

The drugs used were mazindol (5(*p*-chlorophenyl)-2-5-dihydro-3H-imidazo(2,1-α)-isoindol-5-ol) (Sandoz); SKF 39728-A (1-*N*-benzyl-β-methoxy-3-trifluoro-methylphenethylamine) and 4-chloroamphetamine HCl (Smith, Kline and French, Philadelphia, U.S.A.); (+)-fenfluramine HCl and S 992 [trifluoro-methylphenyl(benzoyloxy)-ethylamino-2-propane] (Servier Labs., Paris); phentermine (α-dimethylphenylethylamine) (Pennwalt, Rochester, USA); diethylpropion(2-diethylaminopropiophenone) (Richardson Merrell, Naples, Italy); (+)-amphetamine sulphate (Recordati, Milan, Italy).

Table 1 shows that, as found by Richter & Smith (1974) with human platelets, several anorectic drugs including phentermine, diethylpropion, SKF 39728-A and (+)-amphetamine are not capable of inhibiting 5-HT uptake and neither are they able to release 5-HT from rat platelets *in vitro*, as does fenfluramine.

S 992, a congener of fenfluramine, is inactive as an inhibitor of 5-HT uptake, while it is a weak releaser of 5-HT from platelets. Mazindol is comparable to fenfluramine in its capacity to inhibit 5-HT uptake but it too is a weak releaser of 5-HT. 4-Chloroamphetamine is more effective than fenfluramine on both parameters.

Table 2 shows a dose-response for mazindol and 4-chloroamphetamine on 5-HT uptake. Table 3 shows that both drugs strongly inhibit the initial rate of uptake; the

Table. 1. *Comparison of the effect of different anorectic drugs on ^{14}C-5-HT uptake (after 15 min) and release (after 2 h) from rat platelets.* Figures represent the mean ± s.e. of at least 4 different experiments.

Drug ($2{\cdot}5 \times 10^{-5}$M)	% Inhibition*	% Release**
(+)-Fenfluramine hydrochloride	52·0 ± 2·8	38·5 ± 1·3
(+)-Amphetamine sulphate	<5	<5
S 992	<5	10·5 ± 0·6
SKF 39728-A	<5	<5
Phentermine	<5	<5
Diethylpropion	<5	<5
Mazindol	50·1 ± 1·8	10·0 ± 0·7
4-Chloroamphetamine	70·1 ± 1·1	61·7 ± 1·2

* In respect to the uptake in controls.
** In respect to the 5-HT present in the control platelets at the end of the experiment.