# THE INFLUENCE OF SURFACTANTS ON THE FROG SKIN ION AND WATER PERMEABILITY

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It is well known that sublethal doses of surfactants affect the cellular adhesion in the superficial layers of the exposed epithelia of aquatic animals (1, 2), introducing a shunt in the active transport pathway, and that they are adsorbed by the cellular membranes, interacting with proteins and lipids and influencing the ion end water permeabilities (3). It has also been shown that the electrical behaviour of both the isolated (4) and the “in situ” (5) frog skin is heavily affected and that the isoosmotic, active transport coupled, water flow is abolished (6). We will propose a model for the toxic action of surfactants, based on the analysis of the decay of the electrical potential difference (pd) and short circuit current (scc), which accounts for the mentioned observations.

The pd and scc of the ventral skin in the live, pithed, frog have been measured as described elsewhere (7). After a control period with the animals perfused in normal Ringer at pH 7 (7), the non-ionic surfactant nonil-(oxyethyl)$_9$-phenol (NPO) of mean molecular weight 531 or the anionic surfactant n-dodecil-benzensulphonate (LAS) were added to the solution bathing the external skin surface at concentrations from .1 to 1 and .1 to .5 mM respectively. The osmotic water flow driven by sucrose 0-200 mM added to the external perfusing solution has been measured gravimetrically by the leg skin bag method (8).

The time course of the pd and scc decay induced by NPO and LAS, expressed as percent of the pd and scc values obtained during the control periods, is represented by complex curves which can be approximated by means of a sum of up to four exponentials:

$$V = V_1 e^{-v_1 t} - V_2 e^{-v_2 t} + V_3 e^{-v_3 t} - V_4 e^{-v_4 t}$$

$$I = I_1 e^{-i_1 t} - I_2 e^{-i_2 t} + I_3 e^{-i_3 t} - I_4 e^{-i_4 t}$$

where V and I are the percent values of pd and scc respectively and t is time. The values of the constants for .5 mM NPO are given in the table below

| **i** | **1** | **2** | **3** | **4** |
|---|---|---|---|---|
| $\mathbf{V_i}$ | 117.1 | 132.1 | 332.4 | 217.5 |
| $\mathbf{I_i}$ | 109.7 | 83.7 | 254.2 | 180.2 |
| $\mathbf{v_i}$ | 0.40 | 0.27 | 0.14 | 0.015 |
| $\mathbf{i_i}$ | 0.41 | 0.28 | 0.14 | 0.016 |

The osmotic water flow measured just after the addition of .5 mM NPO to the external perfusing solution is not significantly different from the one measured in absence of the surfactant. Only at zero sucrose concentration the spontaneous water flow is inhibited. This occurs too rapidly to be accounted for by the active transport decay described by the multiexponential functions. It

can be correlated only with the first of the exponential terms, describing the initial increase of both V and I, with practically constant overall skin resistance.

The situation can be discussed in terms of the electrical analog of a double series membrane proposed by Schultz (9), fig. 1. It can be assumed that the first step in the surfactant's action is its adsorption on the external cell membranes of the *stratum corneum*, leading to an increase of $R_2$, that is the resistance of the channels for passive ion transport. This may lead to the initial pd and scc increase and to an uncoupling of ion and water flows, without affecting the osmotic water flow. In effect there is evidence of a substantial osmotic water transport across the lipid bilayer (10). This hypothesis is supported by the fact that LAS has a much slower action than NPO, while preliminary experiments with the cationic surfactant dodecilpyridinium HCl show that this molecule reacts much more rapidly than NPO, indicating that the interaction occur mainly with the negatively charged membrane proteins.

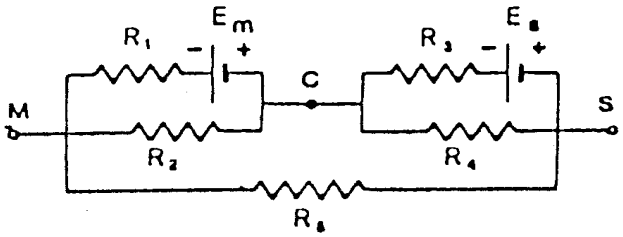


fig. 1

The subsequent step, accounted by the second exponential, may be an impairment of the tight junctions, represented by a decrease of the epithelial shunt $R_5$. This is slower than the $R_2$ increase but, as $R_5$ in "tight" epithelia is much higher than the other resistances (9) it balances the $R_2$ increase, leaving the overall resistance practically unchanged.

In the third step the surfactant is free to diffuse across the impaired tight junctions and to interact with the channels for passive ion transport or the underlying cells, with a consequent increase of $R_4$. This slows down the pd and scc decrease till, in the last step, the active transport processes are impaired either as a consequence of a direct interaction of the surfactant with the transport processes (4) or of a cell membrane disruption, as observed by electron microscopy in fish gills (1, 2).


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