Passive transport across bilayer lipid membranes: Overton continues to rule

According to Overton's rule, membrane permeability (P_M) of a molecule increases with its hydrophobicity. Experiments with a series of carboxylic acids now suggest the opposite: the most hydrophilic acid exhibited the highest P_M (1).

The experiments, however, do not justify this conclusion for the following reasons: (*i*) The reported membrane resistance ($R_M = 1/P_M = 1/2.2 \times 10^{-3}$ cm/s) is much lower than that of near-membrane unstirred layers (UL) ($R_{UL} = 1/P_{UL}$). As every resistance adds to the total resistance ($R = 1/P$) (2), we arrive at

$$\frac{1}{P} = \frac{1}{P_{UL,1}} + \frac{1}{P_M} + \frac{1}{P_{UL,2}}$$

$$= \frac{\delta_{UL,1}}{D} + \frac{1}{P_M} + \frac{\delta_{UL,2}}{D}$$

$$= \frac{20\,\mu m}{1.27 \times 10^{-5}\,cm^2 \cdot s^{-1}} + \frac{1}{2.2 \times 10^{-3}\,cm \cdot s^{-1}} + \frac{2mm}{1.27 \times 10^{-5}\,cm^2 \cdot s^{-1}}$$

$$= \frac{1}{6.35 \times 10^{-3}\,cm \cdot s^{-1}} + \frac{1}{2.2 \times 10^{-3}\,cm \cdot s^{-1}} + \frac{1}{6.35 \times 10^{-5}\,cm \cdot s^{-1}}$$

$$= \frac{1}{6.1 \times 10^{-5}\,cm \cdot s^{-1}}$$

where D, δ_1, and δ_2 denote the diffusion coefficient and the thicknesses of the first and second ULs, respectively. Thus, in case of acetic acid, R_M is only $\approx 3\%$ of R. The value increases to 5% for hexanoic acid. Because this increment is 10-fold smaller than the noise-to-signal ratio, its detection is doubtful. Under conditions where $R_M > R_{UL}$ (pH > pK_a), a P_M of 6.9×10^{-3} cm/s was found for acetic acid (3). In contrast to ref. 1, buffer molecules were present and explicitly considered in the model.

(*ii*) Steady-state equations were applied, although it takes at least 26 times longer than assumed in ref. 1 to reach the steady state. The factor was derived from the half-time of diffusion through the UL (2):

$$t_{\frac{1}{2}} = 0.38 \frac{\delta_{UL,2}^2}{D} \approx 1{,}200\,s$$

If a steady state was present in ref. 1, there should have been some kind of convection that acted to diminish the UL (Fig. 1).

Control experiments (e.g., lowering P_M with cholesterol) would have revealed the above inconsistencies. We conclude that experimental evidence questioning Overton's rule is deficient. Despite continuous debate (4), its impact prevails.

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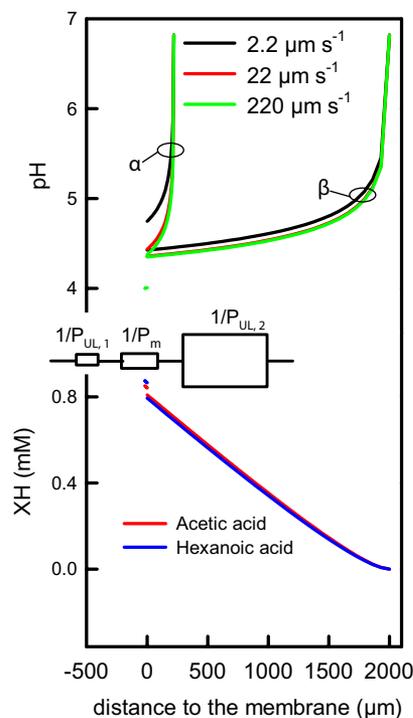

Fig. 1. Theoretical pH (*Upper*) and weak acid (*Lower*) distributions in the membrane vicinity. To show the effect of unstirred layer size, we performed model calculations in which we fixed $\delta_{UL,1}$ to 20 μm and $\delta_{UL,2}$ to 220 (α) or 2,000 μm (β). The calculations represent a minor modification of the previously published model (3). They were carried out analogous to the calculations of bilayer and aquaporin CO_2 permeabilities (5). The differential equations for the combined processes of diffusion and chemical reaction were solved with respect to the boundary conditions (*i*) that pH is equal to bulk pH at the edge of the second (right) unstirred layer and equal to 4.0 at the edge of the first (left) unstirred layer and (*ii*) that total acid concentration (the sum of the anionic and protonated acid concentrations) was equal to 1 mM at the $-20\,\mu$m boundary and 0 at the 220 μm (α) or 2 mm (β) boundary. (*Upper*) The similarities between the theoretical distribution obtained with the smaller $\delta_{UL,2}$ and the experiment of Grime *et al.* (1) indicate that the experiment was not necessarily in the steady state. Because the mathematical apparatus used by Grime *et al.* does not include any time dependence, it does not allow determination of membrane permeability. Varying the permeability coefficient for acetic acid by 2 orders of magnitude revealed only minor changes in the 2-mm-wide unstirred layer. (*Lower*) The theoretical concentration distributions of acetic (P_M = 22 μm/s) and hexanoic (P_M = 6.3 μm/s) acids are very close to each other. In both cases, membrane resistance (1/P_M) to acid flux is negligible.

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