

## Letters to the Editor

### Lateral Diffusion of Protons along Phospholipid Monolayers

In their recent interesting paper on the transport of protons across lipid bilayers by the protonophore S-13 [2], Kasianowicz and colleagues rejected our observation that protons diffuse rapidly along the interface between water and phospholipids when these latter are spread in monolayers [3–5, 7]. They rather suggested that our experimental observation was linked to “a convective flow of lipids which dragged along the unstirred layer that contained protons at a high concentration.” We cannot agree with such a suggestion for the reasons which follow.

The major argument of the authors was the high experimental value of the lipid lateral diffusion coefficient we reported. We knew that this value was high (two orders of magnitude larger than when no stirring was present [1, 8]) but noticed that this was not due to stirring but linked to the vibrations induced by the stirring motor. As reported in refs. 4 and 7, the absence of flow was checked by observing spread talc on the water surface.

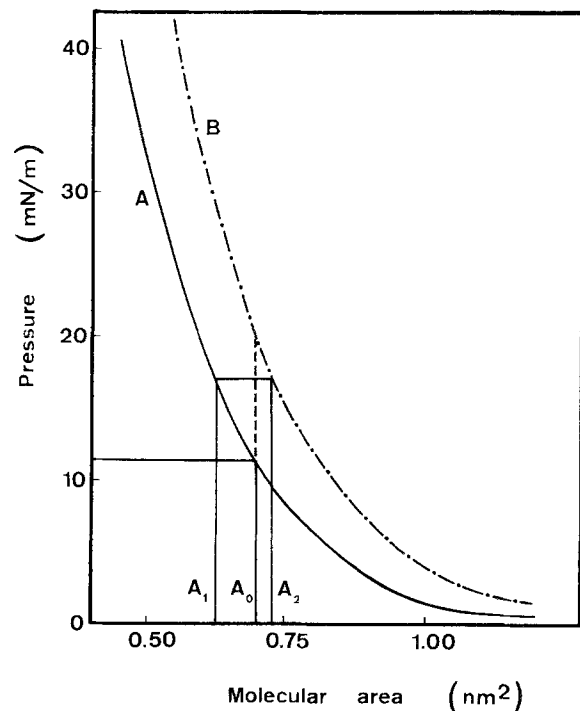
We observed that protons were moving 20 times faster along the interface than in the bulk phase. If one follows the argumentation of Kasianowicz et al., one must accept that the stirring is faster at the interface than in the bulk underneath, although the stirring bar is at the bottom of the trough. This is in fact in contradiction with their description of unstirred layers where “irrespective of how fast one mixes the bulk of a solution, the fluid velocity adjacent to the surface will be low.” We reported that the interfacial pH gradient was very steep [3] and that the bulk phase pH is observed again at a distance of a few nm from the interface. This experimental fact cannot be explained by a convective flow of the unstirred layer which thickness is about 0.1 mm [2]. Furthermore, they implicitly supposed that the pH of the unstirred layer was very low but that the one of the bulk keeps its initial value. The existence of a barrier for protons between the bulk and the unstirred layer was not explained.

We reported that the relationship between distance and time in the proton movement was quadratic [4], that the conduction was sensitive to the strength of the buffer [4] and that the presence of  $\text{Ca}^{++}$  was inhibitory in the case of phosphatidylethanolamine [5]. All these observations are not in agreement with their suggestion.

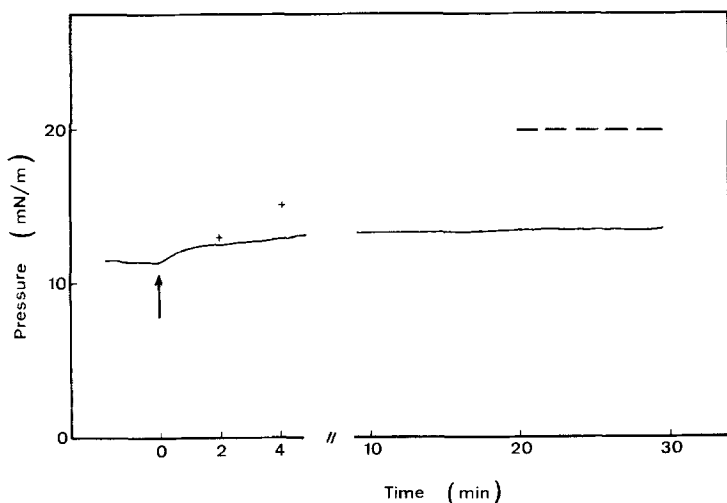
A property of their model would be its ionic nonspecificity. We try to observe the lateral conduction of  $\text{Na}^+$  along phosphatidylglycerol monolayers.

The compression isotherms of acidic phospholipid monolayers are known to be under the strict control of the ionic content of the subphase [6]. In other words, at a constant molecular area, the surface pressure increases with an increase in the ionic content (Fig. 1). We investigated the existence of a lateral conduction in  $\text{Na}^+$  along a PG monolayer by observing the behavior of the film surface pressure after the injection of a NaCl-saturated aliquot in the injection compartment of the trough (see [3–

5, 7] for a description of the experimental set-up). As shown in Fig. 2, no significant change occurred, proving that no fast lateral conduction of  $\text{Na}^+$  existed along the polar heads. If the proposal of Kasianowicz et al. [2] was valid, then the “convective flow of lipids in their monolayer, would drag along the aqueous unstirred layer that contains  $\text{Na}^+$  at high concentration” and an increase in surface pressure should be observed (+ on the graph).



**Fig. 1.** Compression isotherms of egg phosphatidylglycerol (PG) on subphases with different ionic contents. (A) The subphase was pure water (pH = 6). (B) The subphase was 10 mM NaCl (pH = 6). When the monolayer is spread on the A subphase at the  $A_0$  molecular area (surface pressure = 11.5 mN/m), injection of NaCl under the film induces an increase in the pressure along the dotted line. The kinetics of this increase is a direct function of the movement of ions in the subphase. At any time, the surface pressure is homogeneous in the film and is indicative of the ratio of the surfaces of the film being on pure water ( $A_1$  molecular area) and on an ionic containing subphase ( $A_2$  molecular area). When the ions have diffused everywhere in the subphase, a new state of the film is reached on the B isotherm with the  $A_0$  molecular area.



**Fig. 2.** Kinetics of the surface pressure change of the PG monolayer following the localized NaCl injection in the subphase. A PG monolayer was spread on an aqueous ion free subphase at a 11.5 mN/m pressure. At the arrow, an aliquot of a concentrated NaCl solution was added in the injection compartment where a slow stirring was present. The stirring conditions were the same as in our proton experiments. A small increase of the pressure is induced just after the injection, but then the pressure kept an almost constant value. If the convection process which was suggested by Kasianowicz et al. was present, the surface pressure can be computed from the isotherms given in Fig. 1 and from the movement of protons we observed in earlier works; the results are shown (+). Furthermore, under their assumptions, the ionic content of the unstirred layer under the monolayer should be high very rapidly, and the surface pressure should be the one indicated in Fig. 1 (19.5 mN/m) and shown by the dotted line on the right side of the graph

We noticed that our observation of a fast proton transfer along the lipid polar heads is not in conflict with the results of the authors [2]. They wrote that a rapid diffusion of protons in the reaction layer of thickness 1–10 nm is in agreement with the “interesting possibility” of a direct combination of a proton in the aqueous phase with an adsorbed anion.

At the present state of our investigations [3–5, 7], our observations lead us to propose that the fast lateral proton transfer along the interface is due to the existence of a hydrogen-bond network involving the polar head groups and the interfacial water molecules as described in ref. 5.

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## Reply to: Lateral Diffusion of Protons along Phospholipid Monolayers

The letter of Prats et al. [7] contains several misconceptions, which we will not discuss. Instead, we reiterate our claim [3] that even if protons did diffuse rapidly with a diffusion coefficient  $D_1$  in a narrow region of width  $w_1 < 10$  nm adjacent to a monolayer or bilayer, Prats et al. [4–6, 8] would not have detected this rapid movement. We present our argument in the form of a simple equation.

Many experiments (e.g. 2, 3) demonstrate that protons in the aqueous phase adjacent to a bilayer exchange rapidly with protons in the bulk aqueous phase, as expected. If the width of the bulk aqueous phase in the experiment of Prats et al. [8] is  $w_2$  ( $>1$  mm) and the diffusion coefficient of protons in this phase is  $D_2$ , the effective diffusion coefficient they measure for the two parallel regions is (e.g. 1):

$$D = (w_1 D_1 + w_2 D_2) / (w_1 + w_2)$$

provided the protons diffuse a sufficient distance that they equilibrate between regions 1 and 2 over most of the diffusion distance. The protons do diffuse a distance  $>1$  cm in their experiments, which means this steady-state equation is applicable.

Thus we argue their result  $D = 20D_2$  implies either that  $D_1 > 10^6 D_2$  (see equation), which is physically unreasonable, or that their measurement is the result of an artifact, presumably the same artifact that caused their measurement of the diffusion coefficient of lipids in the monolayer to be orders of magnitude too high. Their observation that the apparent rapid diffusion of protons ceases when either the stirring motor is turned off [5] or the monolayer is in a gel rather than a liquid-crystalline state [6] provides a clue to the nature of the artifact.

Of course, our argument does not imply the diffusion coefficient of protons in the water near a membrane must have its normal value. In fact, Yam et al. [9] have recently measured the diffusion coefficient of protons in the water spaces between the bilayers that constitute a multilamellar vesicle. They trapped 8-hydroxy-pyrene 1-3-6-trisulfonate in these spaces, dissociated protons with a short laser pulse, then measured the rate of recombination of protons with the pyrenate anion. Stirring artifacts must be negligible in these narrow (1–3 nm) spaces. They find that the diffusion coefficient of protons in these water spaces is smaller, not larger, than the diffusion coefficient of protons in a bulk aqueous phase.

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