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The ARO grant funded the research of Ms. Susan Richardson for 3 years. She obtained her PhD degree after this time period and is now working for the Environmental Protection Agency.

The publications resulting from her work are given below:

1. F. M. Menger, S. D. Richardson, and G. R. Bromley, J. Am. Chem. Soc. 1989, 111, 6894. "Ion Conductance along Lipid Monolayers".
2. F. M. Menger, S. D. Richardson, M. G. Wood, and M. J. Sherrod, Langmuir, 1989, 5, 833. "Chain-Substituted Lipids in Monomolecular Films. Effect of Polar Substituents on Molecular Packing".
3. F. M. Menger, M. G. Wood, S. Richardson, Q. Zhou, A. R. Elrington, and M. J. Sherrod, J. Am. Chem. Soc. 1988, 110, 6797. "Chain-Substituted Lipids in Monolayer Films. A Study of Molecular Packing".

All three of the above publications acknowledge ARO support. Of the three articles, the second and third are peripheral to the main thrust of our ARO proposal; they resulted as spin-offs from data generated while setting up the film balance/clean room facility. Thus, this Final Report will focus solely on the work reported in the first article which appeared as a communication in the J. Am. Chem. Soc. The results therein occupied about 2 years of the total support period. Much of this time, as delineated in detail in a previous 6-month report, was occupied in securing reproducible data. Measuring the ion conductance in a monomolecular film is, as we had anticipated, an extremely challenging objective and attempted previously by only one other group in Wales. As reported below, our results and those of the British group are not in agreement.

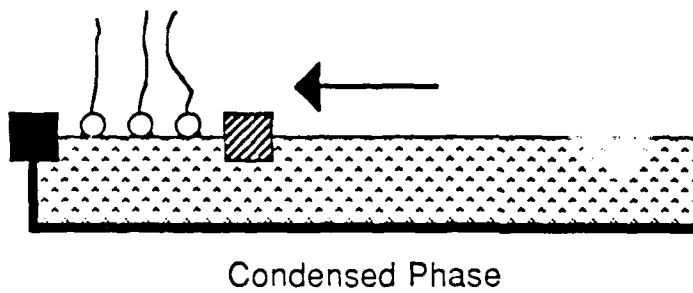
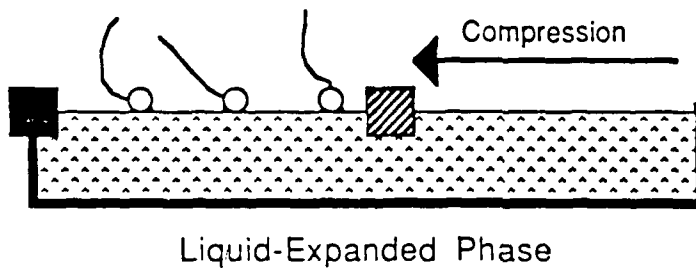
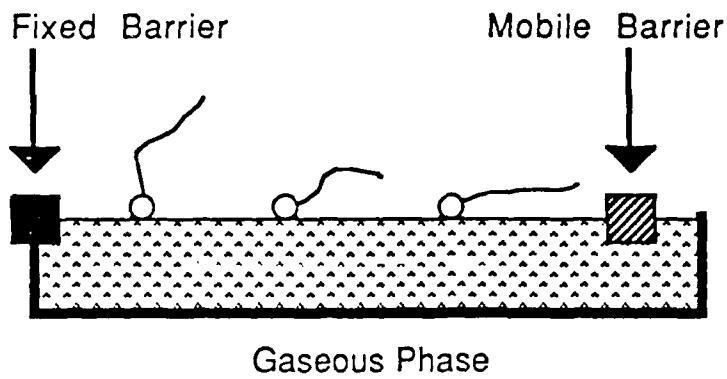
The Basic Experiment

Page 3 shows a film balance set-up. A monolayer of a lipid or related substance is placed upon a subphase of water. In the gaseous state, the molecules move freely with the changes often parallel to the water surface. When the film is compressed, less and less room is available for the film, and as a consequence the chains tend to stand vertically to the water surface. Thus, it is possible to reorient molecules mechanically such that they adopt a gaseous, liquid, and finally a solid-like packing.

The question brought up in our ARO proposal is, "What is the ionic conductivity in films compressed to various phases?" This is an inherently interesting and to date not fully answered question. The question seeks to define a fundamental property of monomolecular films. In addition, the question has taken recently a considerable biological importance. This is explained in the next paragraph.

Peter Mitchell in his Nobel Prize winning "chemiosmotic hypothesis" proposed that proton movement across a membrane creates a potential that drives ATP production.¹ In other words, proton flux develops an electrical potential that represents, in effect, energy storage. The cell is capable of utilizing this stored energy for the synthesis high-energy bonds in the form of ATP.

More recently, others have proposed that protons are not moved across membranes but, instead, along membrane surfaces from one patch to another.



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This idea, implying rapid ion movement along film surfaces, has not yet been adequately tested. Our experiments were set up to do so. The idea was to insert Pt electrodes into the film (as well as the subphase, of course) and to measure the conductance directly as the film was being compressed. If, for example, the conductance increased upon forming an ordered solid film, then one might surmise that the "lateral ion movement theory could have some validity.

The above experiment, although seemingly simple, turned out not to be so. Reproducibility was a serious problem that preoccupied us for literally months. Numerous instrumental and procedural refinements were observed to minimize our problems. These included: (a) Switching from d.c. to a.c. circuitry. (b) Protecting the apparatus from CO_2 by means of N_2 and Ascarite. (c) Employing a second set of reference Pt electrodes which were placed in the subphase outside the film in order to subtract out spurious effects in the subphase. (d) Systematically testing all conceivably variables including fluctuations in temperature, vibrations, water quality, nature of depositing solvent, applied voltage, and lipid impurities. Only after about a year of such laborious testing did we finally obtain results we trusted. These are shown below along with similar experiments carried out concurrently by Morgan³.

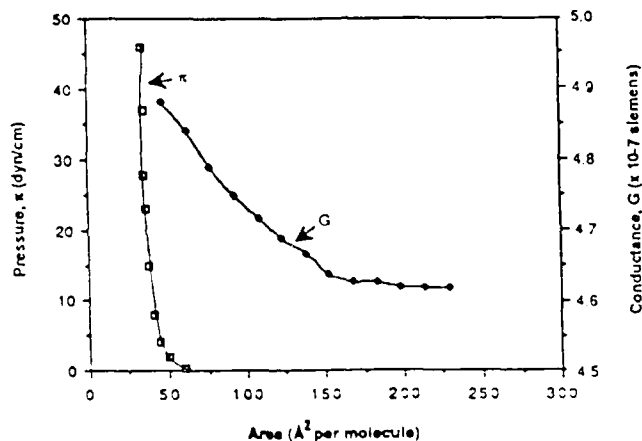


Figure 1. Plots of pressure (π) and conductance (G) vs film area for dipalmitoylphosphatidylethanolamine. Data are taken from Morgan et al.³

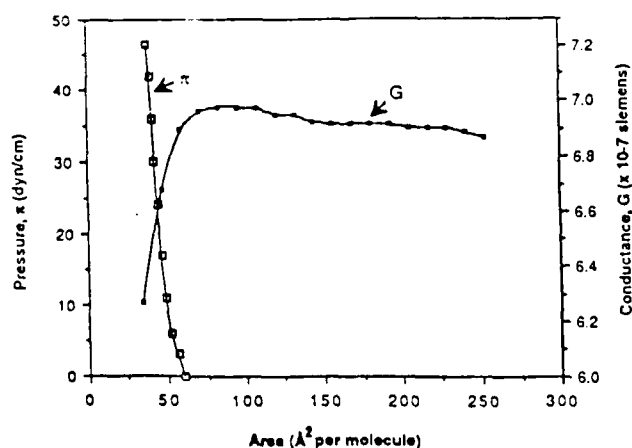


Figure 2. Plots of pressure (π) and conductance (G) vs film area for distearoylphosphatidylcholine at 23.5 °C. Plots were traced simultaneously using ac circuitry for the conductance measurements. Similar curves were obtained in deionized water and 0.5 μM NaCl as the subphase.

Morgan's work (Figure 1) indicates that conductance G increases as the film is compressed (right to left on the X-axis). Intuitively, one might expect this result, reasoning that as the ions pack, their density increases and hence the conductance should increase (much like the conductance increase when the concentration of NaCl in water is elevated). However, Morgan feels, and we agree with him, that it is not the lipid that is carrying the ion current. Instead, adventitious protons (known to be extremely powerful conductance units) constitute the mobile units. According to Morgan, when the lipids are compressed, this changes the structure of the water adjacent to the monolayer in such a manner as to promote proton conductance through that water. Perhaps the water becomes more structured or "ice-like" next to the compressed headgroups of the lipid.

Our data (Figure 2) are in direct conflict with those of Morgan. Film conductance does not change with pressure until, finally, it decreases dramatically when the film becomes "solid". The source of this discrepancy is not clear. We suspect that Morgan is having problems with CO₂ and his d.c. circuitry, but personal communication with the Welch group has not resolved the issue.

Figure 2 is important because it demonstrates, for the first time, that lipid surfaces are not good conduits for ion conductance. This is true even when the head-groups of the lipid are themselves ionic. We feel that the head-groups within the aqueous subphase perturb the water structure so as to impede proton transfer. Naturally, if the film possessed an ion carrier, then ion conductance would be enhanced in such a manner. But a carrier-free film seems unable to act as an ion-conducting device.

Just recently Gutman⁴ et. al. in Israel have provided indirect evidence that we are correct. Spectroscopic data from a laser-excited proton emitter trapped near a membrane surface shows no special propensity of the membrane to conduct. We call this indirect evidence because the experiments required a probe molecule within the membrane, and one must always be concerned about how the probe affects the membranes. With our system, conductance was measured directly with no external probe required. Nevertheless, we are pleased to obtain the support of the Gutman group by a method entirely different from our own.

Figure 2 may seem to be meagre fruit for the amount of effort invested. Actually, however, the experiment is critically important in deciding how biological systems effect energy transduction. It is unlikely that the lateral conductance mechanism is correct, in the absence of an ion carrier, if, as we have shown, a film surface impedes proton flow. Moreover, our data are generally applicable to film chemistry and to the various applications to which films are currently being focused.

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