Phosphatidylserine membranes (either as liquid-crystalline vesicles or as bilayers) in 0.1M NaCl and neutral pH, are very impermeable to cations or anions and exhibit high electrical resistance ($5 \times 10^7 \text{\Omega cm}^2$). However, the same membranes become unstable under conditions of asymmetric distribution of Ca$^{2+}$ or H$^+$: Addition of Ca$^{2+}$ to the aqueous salt solution only on one side of the membrane, produces a lowering of the d.c. resistance, and above a certain concentration results in breakage of the membrane. On the other hand, if Ca$^{2+}$ is present on both sides, the membranes are stable and show very high electrical resistance ($5 \times 10^8 \text{\Omega cm}^2$) over a wide pH range. The above phenomena were not observed with membranes made of phosphatidylcholine. It is suggested that the instability of PS membranes observed in this study is due to the difference in surface energy between the two opposing sides of the bilayer. The biological implications of membrane instability following an asymmetric distribution of Ca$^{2+}$ or H$^+$ is discussed.

INTRODUCTION

Phospholipid membranes have recently become the subject of intensive research as models for biological membrane function (4, 47). Since most biological membranes are complex mixtures composed predominantly of lipids and proteins, artificial membranes composed exclusively of lipids can serve only as very simplified models. However some of the properties of such artificial membranes bear striking similarity to those of the biological membranes (20, 19,
Thus it appears that within certain limitations, artificially produced phospholipid membranes are very promising tools for studying "in isolation" certain molecular events relating to specific membrane functions.

Most of the published work on artificial phospholipid membranes has been performed either with neutral purified phospholipids or with ill-defined lipid-extracts from various natural sources (4, 47). However, it would appear that for studies relating to electrical excitation where binding of ions on fixed charges is an important factor, the phospholipids of choice would be some of the species that carry formal charges (17, 26, 31). Anionic phospholipids (PS, PA, PI, PG, diPG, triPI) are present in most membranes in widely varying proportions, although the percentage of each species within a given membrane is fairly characteristic (51, 3).

Recent work on the surface properties of anionic phospholipids indicates that they bind bivalent metals with high stability constants and even in the presence of high concentration of monovalent salts (2, 13, 26, 5). In contrast, it has been shown that unsaturated PC does not bind appreciable amounts of Ca$^{2+}$ in the presence of 0.1M Na$^+$ or K$^+$ (15, 36, 40, 26, 11).

Studies concerned with the permeability properties of anionic (acidic) phospholipids indicate that these compounds are able to form bilayer structures with very high resistance to the diffusion of ions (17, 28, 34, 33). Membranes composed of PS either in the form of unilamellar vesicles or as planar bilayers are characterized by high electrical resistance, high capacitance and low diffusion of Na$^+$, K$^+$ and Cl$^-$ (34, 31, 21, 22). However, bivalent metals appear to have a pronounced effect on these permeability properties. Thus, it has been shown that Ca$^{2+}$ increases the diffusion rate of Na$^+$ and K$^+$ out of unilamellar PS vesicles (28, 34, 33). It has also been shown that PS bilayers formed in the presence of Ca$^{2+}$ and Na$^+$ are more stable and have higher electrical resistance than those formed in the presence of only NaCl (21, 22).

This intriguing dual role for Ca$^{2+}$ in its ability to produce both an increase and a decrease in the permeability of PS membranes has recently been investigated further by the present authors (31). The data obtained indicate that the stability of a phospholipid membrane is markedly effected by the asymmetric distribution of fixed charges and counterions on the two opposing sides of a phospholipid bilayer.

The present communication is a review of recent data concerning the effect of Ca$^{2+}$ and H$^+$ on the stability of PS bilayers and an account of the biological implications of such a system.