be recovered from *P. berghei* and *P. vinckei*, obtained from mouse and rat erythrocytes, respectively. At least in the case of *P. vinckei*, the activity appears to reside in an enzyme.

These observations suggest the possibility that specific inhibitors of DAD may sometimes have antimalarial activity. The absence of DAD from the mature erythrocyte presumably means that the function subserved by the enzyme cannot be supplied by the host cell. Hence, if an inhibitor blocks the parasite enzyme, the parasite is not likely to escape by obtaining the product of the reaction or of the pathway from the metabolic activity of the infected cell. The effectiveness of inhibitors of DAD as antimalarials is, however, likely to depend also on several other considerations. For one thing, species of *Plasmodium* which have a stable tissue phase or which (like *P. berghei*) preferentially infect reticulocytes (*15*) might well be resistant to such therapy, although it is by no means clear that the level of DAD activity in the reticulocyte is sufficient for the needs of the parasite. Infections with parasites, such as *P. vinckei* or *P. falciparum*, should in theory respond better. Second, of course, the inhibitors of DAD must be specific in two senses. They must not have other biological actions which are toxic to the host animal, and they must inhibit the parasite enzyme to a greater extent than they inhibit the enzyme present in the nucleated cells of the host. Third, the parasite must not be able to trap either the product of the pathway (as uracil or uridine) or orotic acid in amounts sufficient to enable it to mature. Both the normal plasma concentrations of these compounds, and the quantities required for nutrition of the parasite, are not presently known. It is, of course, feasible to inquire systematically into each of these questions, but it is probably more logical to determine directly whether inhibitors of DAD have antimalarial activity. Barbituric acid and 2,4-dihydroxy-6-methylpyrimidine are known to inhibit DAD (*1*). Other inhibitors could presumably be synthesized.

Several tissues, in addition to blood, have deficiencies for enzymes in the synthetic sequence leading to uridine-5′-monophosphate (*16*). Some tissues are also known to be deficient in the sequence leading to cystine (*17*). Finally, the mature erythrocytes of both man and rabbit are unable to perform all the steps in the *de novo* synthesis of inosine-5′-monophosphate (*18*). Perhaps as more organs and pathways are studied, it will be discovered that many mammalian tissues have incomplete sequences with respect to one or another of the small molecules universally found in cells. These enzyme deficiencies, presumably due to cellular differentiation, may occasionally be useful in the diagnosis of infection with intracellular parasites. They may also help in a few instances in devising newer approaches to the treatment of the infection.

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13. The seed stock was provided by Dr. G. Brewer of the University of Michigan Medical School.
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Stability of Asymmetric Phospholipid Membranes

Abstract. *Bilayers (black films) composed of phosphatidylserine are unstable under conditions of asymmetric distribution of calcium or hydrogen ions with respect to the membrane. Addition of calcium ions to the solution (100 millimolar sodium chloride, pH 7.0) on one side only, produces lowering of the direct-current resistance and results in breaking of the membrane. However, with calcium ions on both sides the membranes are stable and show very high electrical resistance.*

Phospholipid membranes in the form of bilayer films or as liquid-crystalline vesicles are currently being used as experimental models for certain aspects of biological membrane transport phenomena (*1*). Such model membranes are promising tools for studying, in isolation, certain molecular events relating to specific membrane function. In most work on bilayers, either neutral phospholipids, such as phosphatidylcholine (PC), or mixtures of phospholipids and other polar lipids of natural origin have been used (*2*). Recent studies on the surface properties of acidic phospholipids (*3*) indicate that these compounds would present an advantage for investigation of electrical excitation, where the binding of ions on fixed charges might play an important role.

Studies with liquid-crystalline vesicles have shown that acidic phospholipids can form stable membranes of low permeability to ions (*4*). Moreover, small concentrations of Ca^{2+} produce a large increase in the diffusion rate of trapped K^+ from vesicles composed of phosphatidylserine (PS) and phosphatidic acid (PA). This increase seemed at first to be contrary to the known effect of Ca^{2+} in decreasing the permeability of nerve cells (*5*). However, recent experiments with bilayer membranes composed of PS indicate that, when Ca^{2+} is present on both sides of the membranes, the resistance is higher than that of the same bilayers without Ca^{2+} (*6*).

These two observations, suggesting two contrasting effects for Ca^{2+}, constituted the starting point for the present study. Our results show that the asymmetric distribution of fixed charges and counter-ions on the two opposing sides of phospholipid bilayers have a profound effect on their stability and electrical properties.

The procedure used in this study has been described (*7*). Purified PS and PC (purchased from Applied Science Labs, Inc.) were prepared, by adaptation of earlier chromatographic procedures, from beef brain and egg yolk, respectively (*8*). The membranes were formed from a solution of the phospholipid in decane (20 mg/ml) with a small amount of methanol (0.5 percent by volume) added to prevent the formation of gel. Aqueous solutions, with twice-distilled (over $KMnO_4$) water, contained NaCl (100 m*M*) at a certain *p*H (adjusted with HCl or NaOH) and $CaCl_2$. The *p*H and $CaCl_2$ concentration of the outside vessel were also changed after the formation of the bilayer by addition of the appropriate solution with continuous stirring.

Phosphatidylserine bilayers can be formed at a variety of *p*H values in 100 m*M* NaCl (Fig. 1). The d-c resistance reaches a maximum at *p*H 3. Above a *p*H of approximately 8, the PS membranes are very unstable and break before the resistance can be measured. Small variations on the absolute values for resistance were observed with different batches of phospholipids, but results were generally consistent. The dependence of the membrane resistance and stability on the *p*H, and consequently on the ionization of polar groups at the surface, is in general agreement with recent calculations on bilayer-micelle transformation (*9*).

Phosphatidylserine membranes were also prepared in the presence of Ca^{2+} (1 m*M* $CaCl_2$ and 100 m*M* NaCl). The *p*H-resistance curve obtained (Fig. 1) indicates a high resistance over a wide *p*H range. Stable membranes with high resistance were obtained, even at high *p*H (9.5), with Ca^{2+} present on both sides at a concentration up to 100 m*M*. Contrary to these results, addition of Ca^{2+} to only one side of the membrane lowers the d-c resistance; above a certain concentration of Ca^{2+}, the membranes break. Typical resistance with PS at *p*H 7.0 (100 m*M* NaCl) is 6.8×10^7 ohm cm^2. After the addition of 1 m*M* $CaCl_2$ on one side, the resistance drops to 2.5×10^7 ohm cm^2. When 1 m*M* $CaCl_2$ is present on both sides, the resistance is 3.4×10^8 ohm cm^2. The concentration of Ca^{2+} necessary to produce breaking is highly dependent on the *p*H. Thus, at *p*H 7.0, in a solution of NaCl (100 m*M*) and ethylenediaminetetraacetic acid (EDTA) (0.1 m*M*), the membranes break at a concentration of 4 to 5 m*M* $CaCl_2$; at *p*H 7.4 they break with 1.4 to 4.0 m*M* $CaCl_2$, and at *p*H 7.8 they break with 0.9 to 1.0 m*M* $CaCl_2$. The EDTA is added in these experiments in order to remove any "indigenous" higher valency metals which are usually extracted along with the PS fraction from natural sources.

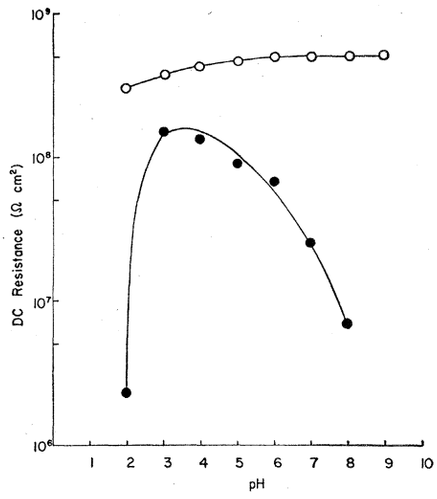

Fig. 1. Specific d-c resistance of PS membranes at different *p*H values; ●, in the presence of 100 m*M* NaCl; ○, in the presence of 1.0 m*M* $CaCl_2$ and 100 m*M* NaCl.

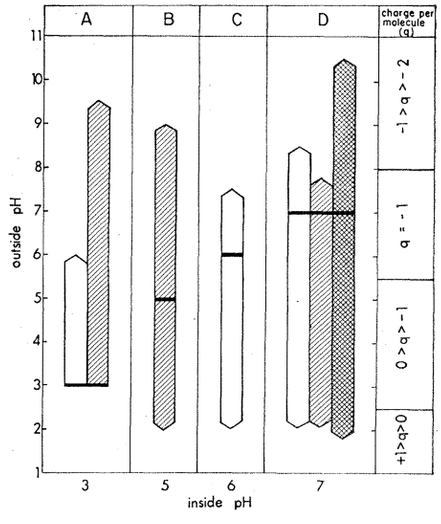

Fig. 2. Stability limits of PS membrane with asymmetric distribuiton of H^+ and Ca^{2+}. Horizontal bars represent the *p*H of the solution at the start of the experiment. The pointed end of each vertical bar represents the *p*H of the outside solution at the point when the membrane breaks. (Open bars) Only NaCl present (100 m*M*); (hatched bars) $CaCl_2$ (1 m*M*) added to the outside solution only; (cross-hatched bars) $CaCl_2$ (1 m*M*) present on both sides of the membrane. The charge per molecule shown on the right of the figure is taken from the titration curves of PS monolayers (*3*).

When EDTA is not included in the above experiments, the Ca^{2+} concentration required for "breaking point" is usually higher (approximately 8 m*M* at *p*H 7.0).

Similar results are obtained when PS membranes are made in solutions containing Ca^{2+} (1.0 m*M*), at *p*H 6.0 to 7.5, and EDTA is added only on one side in order to chelate Ca^{2+}. The membranes break upon the addition of at least equimolar amounts of EDTA. This effect of Ca^{2+} is in general agreement with previous observations that indicated an increase in permeability of PS vesicles at 1 m*M* $CaCl_2$ concentration (*4*).

The Ca^{2+} effect is inhibited by the presence of cholesterol. With membranes made from a mixture of PS and cholesterol (molar ratio 1:2) in a solution of *p*H 7.0 in NaCl (100 m*M*), tris(hydroxymethyl)aminomethane(tris)-HCl (0.2 m*M*), and EDTA (0.1 m*M*), the concentration of $CaCl_2$ for "breaking point" is 9 to 10 m*M*, compared to the concentration of 4 to 5 m*M* needed with PS alone.

Membranes made with PC under the same conditions as above showed no instability within a wide range of *p*H (4 to 8) even at high Ca^{2+} concentration (up to 30 m*M*) on one side only. This result strengthens our conclusions about the effect of specific binding of Ca^{2+} on the acidic groups of PS.

The following experiments demonstrate the limits of stability of PS membranes under asymmetric distribution of fixed charges and the effect of Ca^{2+} in counterbalancing the H^+ by binding to the ionized groups of PS (Fig. 2). When PS membranes are made at *p*H 6.0 in NaCl (100 m*M*) and EDTA (0.1 m*M*), the outside *p*H can be increased only to 7.5 at which point the membranes break. When the outside *p*H is decreased the membranes break at approximately *p*H 2 (Fig. 2C). However, if the original *p*H is 3.0, the membranes become unstable and break when the outside *p*H is raised to 6.0 (Fig. 2A). When the same experiment is repeated after the addition of $CaCl_2$ (1 m*M*) to the outside solution, the membranes are much more stable and break only when the *p*H reaches 9.5 (Fig. 2A).

With membranes made at *p*H 6.8 to 7.0 in NaCl (100 m*M*) and EDTA (0.1 m*M*), addition of 1 m*M* $CaCl_2$ on one side has no effect (no breaking) although the resistance drops by more than twofold. If the addition of Ca^{2+} is followed by an increase in *p*H out-

side, the membrane reaches "breaking point" at *p*H 7.7 to 7.8 (Fig. 2D). Under the same conditions, without Ca^{2+} present, the breaking point is reached at *p*H 8.5 (Fig. 2D).

When the same amount of Ca^{2+} is present on both sides (starting aqueous solution, 100 m*M* NaCl, 1 m*M* $CaCl_2$, *p*H 7.0), the membranes can withstand considerably wider changes of *p*H. The breaking point is reached only at *p*H 10.5 and 1.5 with inside *p*H held at 7.0 (Fig. 2D). With the same salt solution as above, chelation of Ca^{2+} only outside by EDTA makes the membranes unstable at *p*H 7.5 to 8.0, which is a much lower *p*H compared to when Ca^{2+} is present on both sides.

Our results suggest that bilayers composed of PS are very unstable under conditions of an asymmetric distribution of charges or divalent cations bound to the fixed negative charges. Each molecule of PS carries one net negative charge at *p*H 6 to 8, more than one negative charge at *p*H above 8, and less than one negative charge to neutral between *p*H 6 to 2 (*3*). It is apparent (Fig. 2) that difference in ionization of one charge or less per PS molecule between the two sides of the bilayer produces instability and breaking. Asymmetric Ca^{2+} binding at neutral or alkaline *p*H, where one equivalent of Ca^{2+} is bound per PS molecule (*10*), also produces instability manifested as lowering of d-c resistance and breaking. Previous experiments with PS monolayers have shown that considerable changes in surface tension occur after addition of Ca^{2+} or changes of *p*H in the aqueous phase (*3*).

It seems reasonable to suggest that the instability of PS membranes described here is due to the difference in surface energy between the two opposing sides of the bilayer. Calculations of energy differences of the two surfaces resulting from asymmetric distribution of charges (*11*) suggest that it is possible that, under these conditions, molecules or clusters of molecules will "invert" from one side to the other. In doing so they would increase the permeability of the membranes which under extreme conditions reach a breaking point. Moreover, the formation of polymeric assemblies of PS with Ca^{2+} (*3*) may introduce an additional entropy factor which would facilitate the "inversion" phenomenon.

Some of the properties of axon membrane in relation to Ca^{2+} and *p*H (*12*) suggest that the molecular mechanism studied here might be relevant in understanding certain aspects of the nerve excitation process. Recent evidence on the existence of fixed negative charges on the axon membrane (*13*) and the birefringence changes observed during stimulation of nerve (*14*) give some support to the suggestion presented here involving molecular reorientation of specific phospholipid molecules as a response to asymmetric distribution of fixed charges and counter-ions. This suggestion is also easily reconciled with some existing theories on the mechanism of action potential (*15*).

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Neoplastic Transformation in vitro of Hamster Lens Epithelium by Simian Virus 40

Abstract. *Hamster lens epithelium infected with simian virus 40 underwent transformation in vitro and produced tumors when injected into homologous hosts. Undisturbed lens epithelium in man and experimental animals has not been observed to undergo neoplastic change. The virus-induced tumors contained undifferentiated cells that were either polygonal or spindle-shaped. Their origin from lens epithelium seems certain since it is possible to isolate this unique structure free of connective tissue and blood vessels.*

The potentialities of the continuously proliferating lens epithelium for malignant growth have been recognized. Mann in 1947 (*1*) reported epithelial tumors occurring in mice after the subcutaneous implantation of lenses admixed with methylcholanthrene. Von Sallmann *et al.* (*2*) observed that the lens epithelium of rainbow trout maintained on a thioacetamide diet showed invasive proliferation having the characteristics of neoplastic growth. We now demonstrate that lens epithelium infected with simian virus 40 (SV40) undergoes malignant transformation.

The lens epithelium constitutes a population of a single cell type. It can be isolated without contamination by any other tissue elements as the lens is surrounded by a noncellular laminated capsule. The epithelial cells lie beneath the capsule and form a single layer covering the anterior surface of the lens. Near the equator the cells elongate and differentiate to form lens fibers. Their nuclei are no longer capable of division, and the fibers ultimately become anucleate. This pure, well-defined cell population is a useful model for studying viral neoplastic transformation, and particularly for observing the role of the viral genome in determining tumor morphology.

Lenses were obtained from the eyes of six 4-week-old Syrian hamsters. The capsules with attached epithelium were aseptically removed by microscopic dissection, cut into explants of approximately 0.5 mm^2, and used to establish cultures in six 2-ounce prescription bottles (*3*). All cultures were incubated at 37°C in medium No. 199 with 20 percent fetal bovine serum. The fluid was changed three times weekly. On the