LETTER TO THE EDITOR

Lenses and the Compression of Black Lipid Membranes by an Electric Field

Dear Sir:

White (1974) and Crowley (1973) have recently referred to the determination of Young's modulus for black lipid membrane elasticity based on the increase in the steady-state specific membrane capacity which results when a potential is applied across the membrane. In these determinations it is assumed that the deformation of a patch of membrane in response to the compressive force is a decrease in thickness and an increase in area with no change in the volume. However, while we agree that the forces acting are too weak to affect the densities of the materials present, these forces are also too weak to change the distance between adjacent lipid molecules in the membrane. This can be shown from the magnitude of the membrane tension change measured in contact angle studies (Haydon and Taylor, 1968; Requena, 1974). Thus the area of the patch must be effectively constant. Haydon (1970) has estimated that the Young's modulus corresponding to a model in which the area per molecule is increased at constant volume would be about $2 \times 10^7 N/m^2$ which is two orders of magnitude larger than the apparent values observed (cf. White, 1974). There is a ready explanation for this discrepancy.

The apparent steady-state specific capacity of membranes formed from either glyceryl monooleate or egg lecithin in *n*-alkane solvents increases with the applied field to an extent which varies with the chain length of the solvent used (Andrews et al., 1970; Fettiplace et al., 1971). The apparent specific capacity is defined as the capacity of the membrane, C, divided by the total apparent membrane area, A_T. Thus for glyceryl monooleate+*n*-decane membranes and 0.1 M NaCl, the apparent specific capacitance increases from 3.86 to 4.12 nF/mm^2 under 100 mV applied potential while over the same range of potentials the capacitance for glyceryl monooleate+*n*-hexadecane membranes is constant at 5.85 nF/mm^2. These and similar more extensive results have been interpreted (Andrews et al., 1970) as implying that the increase in specific capacity under compression for membranes made with *n*-decane is a result of a decrease in thickness of the membrane and that this change in thickness is accomplished by a proportional decrease in membrane volume through the partial exclusion of decane molecules from the membrane into small lenses[1] and into the plateau border which surrounds the membrane. Membranes made with *n*-hexadecane are much thinner because they contain much less solvent, and as a result they are much less compressible.

Following the application of a 100 mV potential across a glyceryl monooleate+*n*-decane membrane, the measured total capacitance increases with a time course which cannot be fitted with less than two time constants. After an initial period of 10–20 ms during which no change is observed ($\Delta C/C < 1\%$), there is a large increase with an apparent time constant of ca. 100 ms and a subsequent slow, smaller increase over a period of 1 or 2 min. In keeping with the explanations offered above, the larger relaxation corresponds to the sum of the effects of

[1]These lenses, which are like isolated fragments of the volume phase contained in the Plateau border, should not be confused with fluctuations in the thickness of the equilibrium membrane structure (Vrij, 1968).

both the thinning of the membrane with exclusion of solvent into submicroscopic lenses and the creation of new membrane at the expense of the plateau border. The increase in the area of the membrane can vary from 100% for membranes initially less than $^1/_{10}$ of the area of the hole in the support to less than 1% for the largest membranes possible in the same cell. The slower change in the total capacity and in the apparent specific capacity is supposed to result from a reduction in the total area occupied by the lenses formed during thinning. These fuse with one another so becoming visible and finally coalesce with the border.

Further evidence for this interpretation is available from observations of the lenses. Immediately after the formation of a large membrane from either glyceryl monooleate or egg lecithin in *n*-decane it is possible to see in reflected light small lenses of the hydrocarbon phase trapped in the membrane[2] (Andrews and Haydon, 1968; Fettiplace et al., 1974). The lenses eventually coalesce with the border, more rapidly if the membrane is held vertically. If a potential of 100 mV is applied across the membrane after formation, small lenses either appear or if already present become much more numerous over a period of 1 or 2 min. By direct observation, the visible lenses occupy only a small fraction of the membrane area. Thus it is not surprising that even before the lenses have disappeared, the ratio of the measured total membrane capacity to the geometrical area of the membrane reaches a constant value, 3.86 ± 0.03 nF/mm^2 (within 1 or 2 min).[3]

It is possible to demonstrate that lenses have a negligible effect on the specific capacity determined for monoglyceride membranes which are more than a few minutes old. When a field is applied to the membrane the tension must decrease. Qualitatively the change may be described as a result of the increase in lateral repulsion along the surfaces of the membrane as the charge stored in the membrane capacity is increased. The change occurs in the absence of detectable thinning and is in no way related to interdigitation of the lipid chains except in that avoidance of chain overlap maintains the membrane thickness (Taylor and Haydon, 1966; Andrews et al., 1970). The magnitude of the tension change $\Delta\gamma_m$ is related to the applied potential ΔV and the true specific capacity (C/A_m) by the Lippmann equation (Parsons, 1954) as adapted for use with a black lipid membrane (Requena, 1974),

$$\Delta\gamma_m = 0.5\,(C/A_m)\,\Delta V^2.$$

The change in membrane tension may be determined accurately by measuring the contact angle between the membrane and an interface of either the plateau border or a large lens since the membrane tension and the constant tension of the border interface, γ_b, are related by

$$\gamma_m = 2\gamma_b \cos\theta.$$

From the slope of the straight line which is obtained when $\Delta \cos\theta$ is plotted against ΔV^2 ($\Delta V < 50$ mV) (Fig. 2), and using the value of the interfacial tension as determined by drop volume measurements (Requena, 1974), the capacity per unit area of black membrane is found

[2]The lenses may be seen more easily under dark-field illumination.

[3]The boundary of the geometrical area was determined from the extrapolated profile of the Plateau border as determined by optical interference measurements (see Fig. 1). In practice the boundary may be taken to be between 1 and 2 μm inside the first bright fringe. Thus even if the first bright fringe is taken as the boundary the error in the geometrical area of a circular membrane will be less than 2% provided that the diameter of the membrane is greater than 200 μm. The specific capacitance was calculated from the slope of the linear relation between membrane capacity and membrane area.

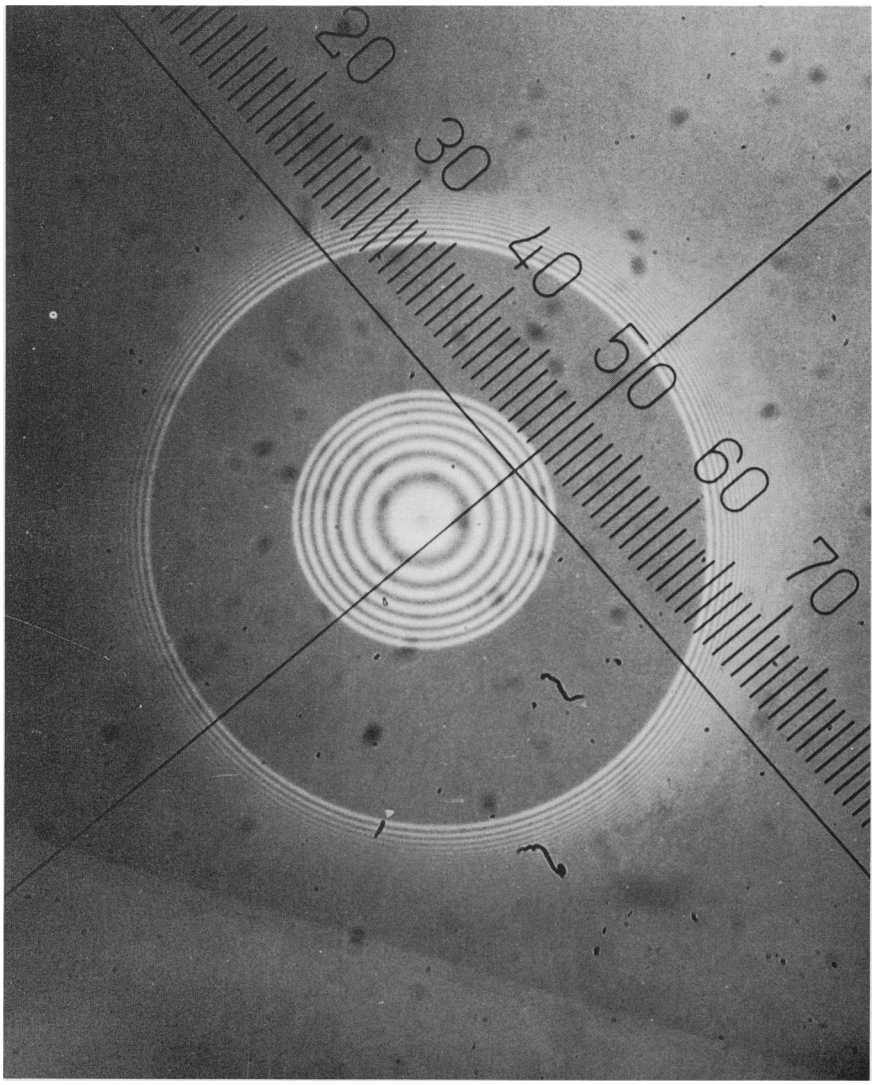

FIGURE 1 A photograph of a glyceryl monooleate+*n*-octane membrane, the surrounding plateau border and a large trapped lens taken using monochromatic reflected light (mercury green line, 546.1 nm). From the spacings of the interference fringes, it is possible to construct the profiles of the border region and of the lens (Requena, 1974).

to be 3.81 ± 0.07 nF/mm^2 for glyceryl monooleate+*n*-decane and 0.1 M NaCl. Since this value is the same as that determined by conventional techniques, the geometrical area and the black membrane area are the same to within 2% and the area occupied by lenses may be ignored.

While the effect of trapped lenses on the measured specific capacity is small, their influence on other types of measurements can be considerable. Thus Pagano et al. (1972) found much more solvent in monoglyceride membranes by a sampling procedure than could have been present in the black portions of the sampled membranes and attributed this result to the effect

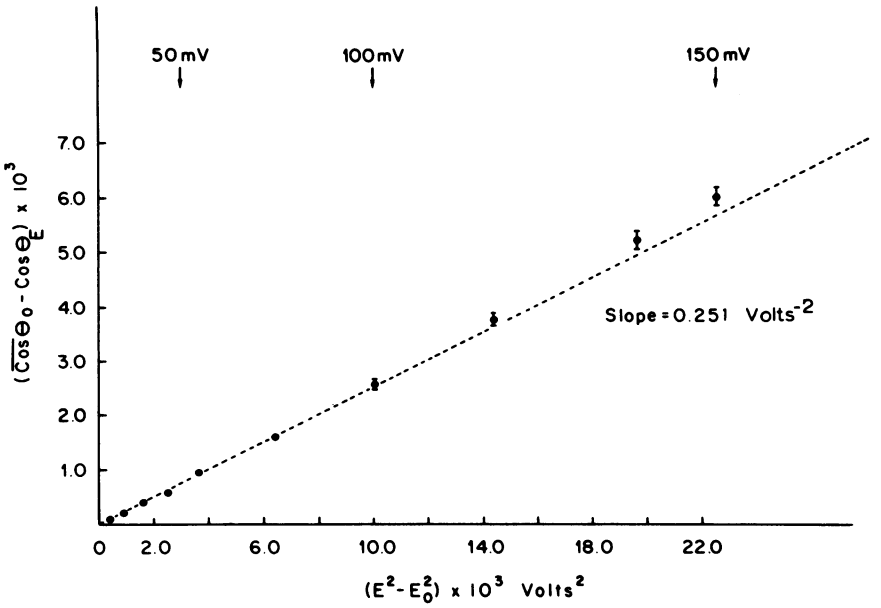

FIGURE 2 The change in the cosine of the contact angle, cos θ, measured for glyceryl monooleate+*n*-decane membranes in 0.1 M NaCl (20°C) as the applied potential, $\Delta V = E$, is increased from 0 to 150 mV (E_0 = electrode potential < 0.1 mV) (Requena, 1974).

of trapped lenses. Similarly, Pagano et al. (1973) have concluded that lenses introduce a substantial error into the reflectance method for measuring the thickness of these membranes.

The observation that lenses become more numerous at high applied fields is a clear indication that the steady-state compression of a black lipid membrane does not occur at constant volume. Thus any attempt to determine a modulus of elasticity from the steady-state data is in error. The true elastic response of the membrane must be sought in the change of capacitance sufficiently soon after a change in applied potential that the composition of the membrane has not had time to alter. For glyceryl monooleate+*n*-decane or *n*-hexadecane the fractional capacity change which occurs faster than that already assigned to solvent exclusion is less than 1%.

Wobschall (1972) using a sophisticated bridge circuit has observed that the change in capacity of hexadecyltrimethylammonium chloride+cholesterol+dodecane membranes occurs with a small, fast component (0.01%) and a larger, slow component ($\tau \sim 100$ ms; 1%). He suggested that the first was due to elastic thinning and the second to a change in the area occupied by submicroscopic lenses. From the discussion above it should be clear that at least part of the slow relaxation could be solvent extrusion. The Young's modulus calculated from the fast change is about 10^7N/m^2.

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