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# STEP CONDUCTANCE INCREASES IN BILAYER MEMBRANES INDUCED BY ANTIBODY-ANTIGEN-COMPLEMENT ACTION

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## Summary

A sharp rise in the electrical conductance of lipid bilayer membranes was observed following the addition of antigen (bovine serum), antibody (rabbit anti-bovine serum), and complement to the neighboring aqueous phases. At low concentrations, step increases in the conductivity occurred which are consistent with the appearance of about 2.2 nm holes in the membrane. Probably attack or lysis of the lipid bilayer by complement is responsible.

Increases in the electrical conductance of lipid bilayer membranes upon the addition of an antigen and antibody to the adjacent solutions were observed by del Castillo et al. [1] and confirmed by Barfort et al. [2]. Antigen is injected into the aqueous phase on one side of the membrane after formation and the corresponding antibody is injected into the aqueous phase on the opposite side. Barfort et al. found that complement was required while del Castillo did not. Shortly after injection a conductance increase occurs, apparently due to small holes in the membrane produced by the attack of complement. The process is similar to the lysis of cells by complement.

The mechanism of complement attack on lipid membranes in vesicle form (liposomes) has been studied in considerable detail by a number of investigators, notably Kinsky [3], but the nature of the lesion has not been fully clarified [4]. Determination of the conductance change with time can provide information on the size of the conducting channel and its rate of formation or opening, as studies of membrane conductivity induced by excitability inducing material [5,6] and gramicidin A [7] have demonstrated.

Conductivity was measured with the apparatus sketched in Fig. 1. Voltage is clamped to a given value, (typically $v_m$ = 20 mV) and the membrane

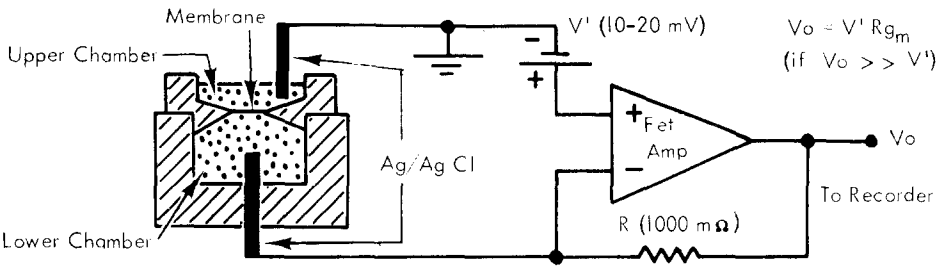


**Fig. 1. Membrane conductance apparatus. Antigen is injected into the saline solution in the lower chamber and then antibody and complement are injected into the upper. The output of the amplifier is proportional to the subsequent conductance increase.**

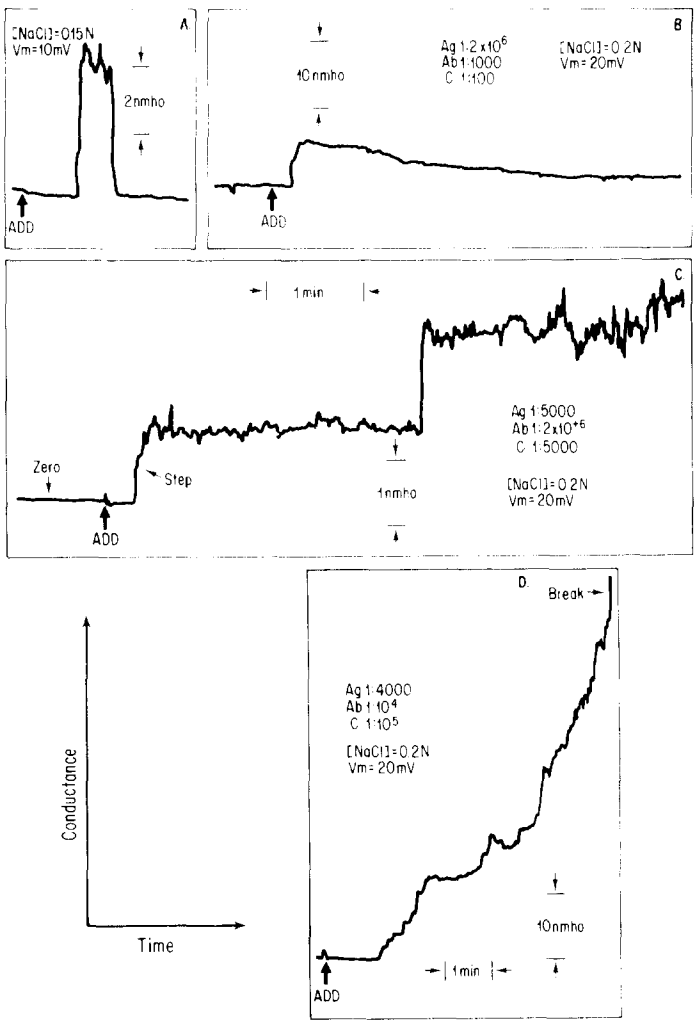


**Fig. 2. Time course of membrane conductance. Response following low levels of antibody (Ab) + antigen (Ag) + complement (C) injection are shown in A, B, C, while D is for higher levels. In A a step conductance increase is followed by a step return to base level. In B the return is gradual. In C two steps are each followed by a fairly steady conductance. In D the rise continues until membrane rupture.**

current, which is proportional to conductance, displayed on a time base recorder. The actual instrument was considerably more elaborate than shown and employed circuits for monitoring membrane capacitance [8] and automatic zeroing of membrane conductance before antibody is added. Rudin-Mueller membranes (area $\approx 0.003$ cm$^2$) were formed in the usual manner [9] from sphingomyelin (10—20 mg/ml) dissolved in a tocopherol (30%)/chloroform(40%)/methanol(20%) solvent. Usually cholesterol ($\approx$10 mg/ml) was added. The aqueous phase consisted of (0.02 to 0.3 M) NaCl in triple distilled water with histidine buffer (0.001 N, pH $\approx$ 7.4) and divalent ions ($10^{-4}$ M $Mg^{2+}$, $Ca^{2+}$). Temperature was held at 37°C. Antigen was bovine whole serum added to the lower chamber (1.4 ml) after the membrane had thinned to the bilayer stage (as indicated by capacitance measurements). Antibody was rabbit antiserum against bovine serum added to the upper chamber (0.25 ml) after the antigen addition. Final antibody and antigen serum dilution ratios varied from $1:10^3$ to $1:10^7$. Guinea pig complement was also added (final dilution $1:10^2$ to $1:10^5$) although not necessary since the rabbit complement was active.

No conductance increase was observed if complement (and antiserum), was inactivated by heating (56°C for 0.5 h), or was added on the side opposite the antibody. Further, little or no reaction occurred if proteins or sera other than bovine were added. These results suggest that (1) complement is required, (2) the antigen diffuses through the membrane to react specifically with the antibody and (3) that the antibody and complement (all components) cannot diffuse completely through. Antigen can be placed on either side of the membrane but the membrane stability is less if both antibody and antigen are on the same side.

Recordings of the conductivity as a function of time after antibody-antigen-complement addition are shown in Fig. 2. After a delay, probably caused by diffusion (chambers are not stirred), the conductivity increases. At high antibody-antigen-complement concentrations the delay was short, the conductance rise rapid, and membrane rupture followed quickly. At lower concentrations step increases in conductance were observed. Usually the onset of conductivity was marked by a step and often other steps followed. As indicated in Fig. 2, the time course of the conductivity increase was variable and a gradual return to the base level sometimes occurred. The onset of conductivity was always accompanied by increased noise. Occasionally noise and steps were seen membranes without added antibody, antigen, or complement for a period preceding spontaneous membrane rupture; but this prerupture noise was rare, more erratic than the antigen-antibody-complement steps, and was greatly reduced by the addition of cholesterol.

By analogy with excitability inducing material and gramicidin studies the step increase is interpreted, following Bean [5], as the formation of a single channel or pore extending through the membrane. We have categorized the observed conductance changes in two groups: steps and noise. Plots of the mean step sizes at 20 mV as a function of salt concentration is shown in Fig. 3. The approximate proportionality to solution conductivity is evidence for the presence of pores. The solid line indicates the conductivity expected for 2.2 nm diameter cylindrical hole filled with sodium ions. Free solution mobility and a 5.0 nm thick membrane are assumed.

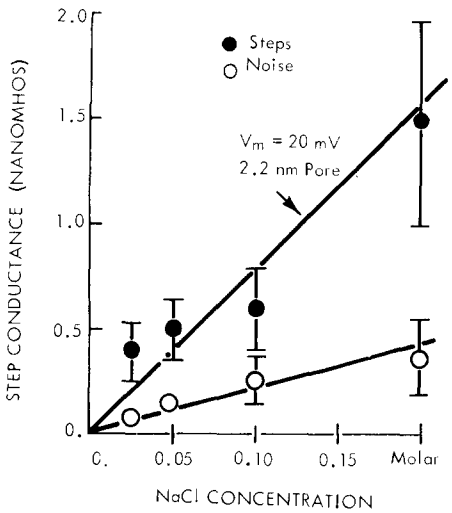


**Fig.3. Mean step conductance as a function of salt concentration. About 100 steps were analyzed.**

Unlike excitability inducing materials or gramicidin A, the step size here is not uniform. This suggests that the pores produced by complement attack are not uniform but rather have a statistical fluctuation, roughly a factor of two about a mean pore size. Variability in the lesion [4,10,11] is not unexpected since the complement complex which attacks the membrane may have a variable number of subunits (especially $C_9$).

Probably the noise, which on some traces has the appearance of minor steps, is a fluctuation in the pore area rather than an opening and closing of a smaller pore since the noise usually follows the onset of steps. Possibly the fluctuation is associated with a variation in the number of complement subunits in the membrane lesion.

A rate of pore opening and closing faster than about one step change per second would not be seen on this experiment because of a limitation on amplifier response time. An average pore area would be observed if the switching rate is rapid and therefore a maximum pore size cannot be inferred with certainty. It is interesting to note that del Castillo et al. [1] report a transient increase in conductivity and Barfort et al. [2] report a steady state value while we find that both can occur.

We feel the bilayer conductivity technique may prove useful in studies of the mechanism of complement attack and, as previous workers have pointed out, can be applied to the rapid determination of antibody or antigen concentration.

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