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EFFECT OF COLLOIDAL SILICIC ACID ON LECITHIN BILAYERS

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SUMMARY

Neutralized solutions of alkali silicates strongly decrease the resistance of black lecithin leaflet membranes. This effect depends on the time which has elapsed since the neutralization of the silicate and reaches a maximum after 4–6 h. Silicate solutions brought to pH lower than 4 have no influence. It seems probable that an oligomeric silicic acid is optimal in decreasing leaflet resistance: the monomer, immediately after neutralization, and the high polymer, after much time has elapsed, are much less effective.

The effect increases with silica concentration. Above $1 \cdot 10^{-4}$ M colloidal silicic acid the leaflets become coloured and thick even if they have been black and thin before the silicate was added. K^+ transference increases in this case.

A possible connection between these results and clinical silicosis can be envisaged.

INTRODUCTION

In course of (as yet unpublished) experiments on the influence of tetrabutyl ammonium salts on lecithin bilayers we observed irregularities which we attributed to the presence of impurities in the tetrabutyl ammonium solutions. They often had been prepared from the hydroxide, which attacks glass, so that it was possible that some silicate may have remained in the solutions, which at first had been purified only by filtration.

Accordingly experiments were begun with dilute water–glass solutions. It turned out that these solutions decreased the leaflet resistance by orders of magnitude. Therefore, pure potassium silicate solutions were prepared from purified silica gel and KOH, and work was resumed with this material as additive to the neutral KCl solutions.

MATERIALS AND METHODS

Lecithin was prepared from fresh eggs by the method of Dawson[1] and was used in 1.5% decane (B.D.H.) solution. The leaflet membranes were smeared on the fresh cut edge of a polyethylene tube of 1.7 mm internal diameter. The tube was changed every day. It was attached to a small cell (about 1 ml) carrying an electrode and a connection to the pressure regulating syringe. This small cell was placed above the liquid level of an outer compartment into which only the polyethylene tube was immersed. The second electrode was placed in the outer compartment. A tube at the bottom of the compartment allowed its contents to be drained and exchanged for another solution, if necessary. The cells were insulated and put on a heating plate and temperature was maintained between 37–39 °C.

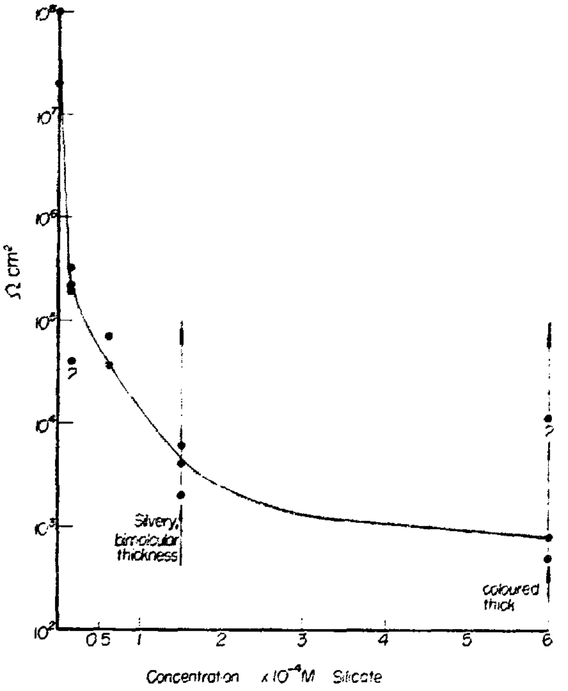
Two Keithley 610B electrometers were used to measure current and voltage, respectively, and a potentiometer was used to apply desired voltages in both directions. The electrodes were Ag–AgCl wires in capillaries filled with 2 M KCl agar.

Capacity was measured using an AVO Type I universal measuring bridge with a 1000 Hz internal source and a Hewlett–Packard Model 400D A.C. V.T.V.M. as zero instrument.

RESULTS

The resistance of lecithin membranes without additives in 0.1 M KCl was between $1 \cdot 10^7$ and $1 \cdot 10^8$ $\Omega \cdot cm^2$. After addition of $2 \cdot 10^{-5}$ M silicic acid at pH 6.5 the resistance decreased to 10^4 $\Omega \cdot cm^2$ and continued to decrease with increasing silica concentration (Fig. 1).

It was found, however, that the decrease of resistance not only depended on the concentration of colloidal silicic acid, but that it also depended on how much time has passed since neutralization of the originally alkaline (pH 12) silicate solution: It is known, that polymerization of the polysilicic acid needs time[2] and its influence on lecithin bilayers evidently depends on its degree of polymerization. The influence



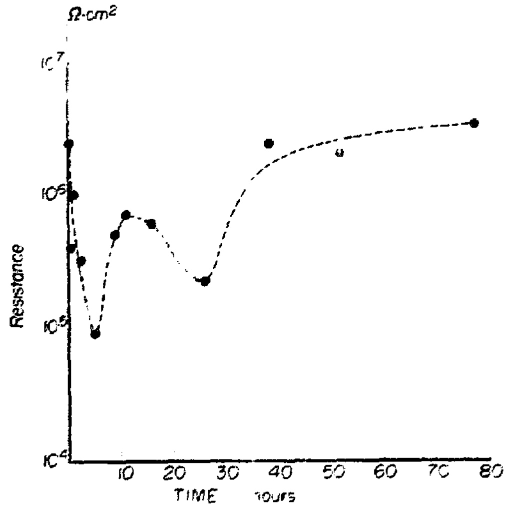


Fig. 1. Resistance of a bimolecular lecithin membrane as a function of the molarity of the silica sol added to the 0.1 M KCl on both of its sides, pH 7. Time elapsed since neutralization of the silicate solution was 5 h.

Fig. 2. Resistance of a bimolecular lecithin membrane between solutions containing 0.1 M KCl and $6 \cdot 10^{-5}$ M silica sol at pH 7 as a function of the time elapsed since neutralization of the silicate solution.

of silicic acid on membranes, also depends on the pH of the silica solution. This again may well be due to its degree of polymerization[2]. It seems, therefore, that a medium degree of polymerization is optimal in influencing the electrical properties of lecithin bilayers.

Fig. 2 shows the resistance of lecithin membranes as a function of the time elapsed from bringing the $1.5 \cdot 10^{-2}$ M silicate solutions to pH 7. The silicate concentration in the 0.1 M KCl of the cell was $6 \cdot 10^{-5}$ M in this experiment. The second minimum of the curve seems to be real; it was observed in several series of experiments at different pH values.

The pH at which the silicic acid was aged had an influence on its properties. This seems natural in view of the fact that polymerization of silicic acid is pH dependent[2]. Below pH 4 we found no influence on leaflets. The resistance of lecithin bilayers decreased more with freshly neutralized silica solutions when their pH was higher, but with these more alkaline solutions it took more time to reach the minimum resistance on the curve, *e.g.* 30 min after neutralization the membrane resistance was $4 \cdot 10^6$ $\Omega \cdot cm^2$ at pH 5.5, $1 \cdot 10^6$ at pH 7 and $4 \cdot 10^5$ at pH 8.5. The lowest resistances were reached at pH 5.5 after 4–5 h ($1 \cdot 10^4$ $\Omega \cdot cm^2$), at pH 7 after 4–5 h ($1 \cdot 10^4$) and at pH 8.5 after 10–12 h ($5 \cdot 10^4$).

At the three pH values mentioned above the resistance rose to $1 \cdot 10^6$–$4 \cdot 10^6$ $\Omega \cdot cm^2$ a day after neutralization of the silicate and there is no further change after that.

Evidently, the situation is quite complicated. The complication must be due to the fact that a medium degree of polymerization of the silicic acid has the maximal effect on bilayer resistance. The time necessary to reach this degree of polymerization is of course pH dependent[2].

The effect of colloidal silicic acid on lecithin bilayers depends on whether the silica was added to one or to both sides of the membrane. Several comparative experiments have been performed, and it was found that the effect was always much larger when both sides of the leaflet membranes were in contact with the silicic acid. For example $1 \cdot 10^{-4}$ M silicic acid was used in 0.1 M KCl, 4 h after having neutralized the potassium silicate to pH 7. With silica present on both sides of the lecithin leaflet resistance decreased from $4 \cdot 10^7$ $\Omega \cdot cm^2$ to $2.5 \cdot 10^3$. When silica was present on one side only, resistance only decreased to $8 \cdot 10^4$. This may perhaps be taken as an indication that colloidal silica crosses the lecithin layer only slowly or perhaps not at all. If it would get through quickly, its steady-state concentration would be similar on both sides of the membrane before it diffuses away into the solution not containing any silica and one would expect the same influence as when it was applied to both sides. The fact, that it exerts so great influence at so low concentrations certainly shows that it must be strongly adsorbed into the membrane surface and would diffuse away slowly. It seems to deform the hydrophilic surface of the membrane with which it comes into contact and thereby opens it for the ions of KCl.

It is also worth mentioning that the black lecithin bilayers become first silvery, when the concentration of silica exceeds $1 \cdot 10^{-4}$ M and capacity measurements showed that the membranes became thicker. They are not bilayers any more. At even higher concentrations of silica the membrane regains its interference colours, even if it had been black before the silica was added to the KCl solution. In an interesting paper on the influence of polylysine on lipid leaflet membranes, Montal[3] also states that the membranes became silvery, with coloured lenses on their surface, while their resistance decreased by 2–3 orders of magnitude. In such cases it might be important to determine by capacity measurements whether the layers are still bimolecular or whether their thickness has increased.

The concentration potential between 0.1 M and 0.01 M KCl solutions (normally about 25 mV, corresponding to $t_K=0.7$) is not changed in the presence of silicate as long as the membranes remain black. However, when the concentration of the silica was high enough ($6 \cdot 10^{-4}$ M) to make the membranes coloured and thick, the concentration potential rose to 43–48 mV. This is equivalent to $t_K=0.9$.

The biionic potential between 0.1 M KCl and 0.1 M NaCl solutions was also examined on black and coloured membranes in the presence of silicic acid between $1 \cdot 10^{-5}$ and $6 \cdot 10^{-4}$ M. No biionic potential was observed, that is there is no difference between sodium and potassium transference.

The breakdown voltage of the lecithin membranes was 250 mV in the presence of $1 \cdot 10^{-4}$ M colloidal silicic acid.

A few tentative experiments on water permeability of the lecithin membranes in the presence of silicic acid showed that water permeability increased by about 40%. The measurements were made as described by Graziani and Livne[4] using sucrose to establish an osmotic gradient.

Silicosis is a chronic illness which occurs among people who work for many years in an atmosphere containing fine crystalline silica or silicate powder. It may be worth considering the above results in this context even though colloidal silicic acid does not seem to give rise to the clinical effect.

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