PHOSPHATIDE MEMBRANES

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AN improved understanding of the effects of drugs on cell membranes is likely to follow from the development of realistic models of these membranes. Many attempts have been made but none of the models so far devised have borne much resemblance to the original.

The reaction which produces a cell membrane can be considered as a fundamental process of biochemistry. It seems likely that this reaction is the precipitation of a phosphatide film from the intracellular fluid by calcium and magnesium salts in the outer liquid, and it is significant that the calcium salt content of intra-cellular fluid is extremely small. This initial phosphatide film is then presumably strengthened by deposition of protein and polysaccharide layers. The membrane so formed has a certain mechanical strength but its most important property is its limited permeability to salts and other substances.

The formation of a complete cell membrane is obviously a complex process involving a number of components. It would be a useful advance if this process could be simulated using a limited number of purified substances of known structure and this is a problem which we have been studying for some time. Our early work showed that films of measurable mechanical strength could be formed at the boundary between a phosphatide sol and a salt solution of concentration sufficient to precipitate the sol[1]. Improved chromatographic methods for purifying lecithin showed that the presence of lysolecithin in lecithin preparations had a considerable effect on the properties of the sols[2].

The next stage in the investigation has been an attempt to produce phosphatide films having limited permeability to salts. Once this has been achieved there exists the possibility of obtaining and studying

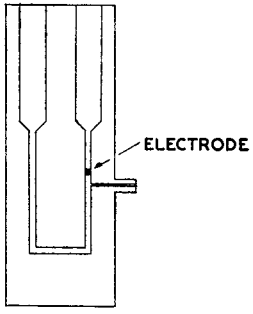

FIG. 1. Diffusion cell.

polarised membranes, a field which should be of interest in the study of nerve impulse and memory storage mechanisms.

Experimental. To study membrane permeability effects the simple perspex apparatus shown in Figure 1, has been used. In this exploratory work, permeability to the precipitating salt only, has been examined. In an extensive series of experiments, boundaries have been formed between a calcium or magnesium chloride solution, usually 10^{-3} N, and a

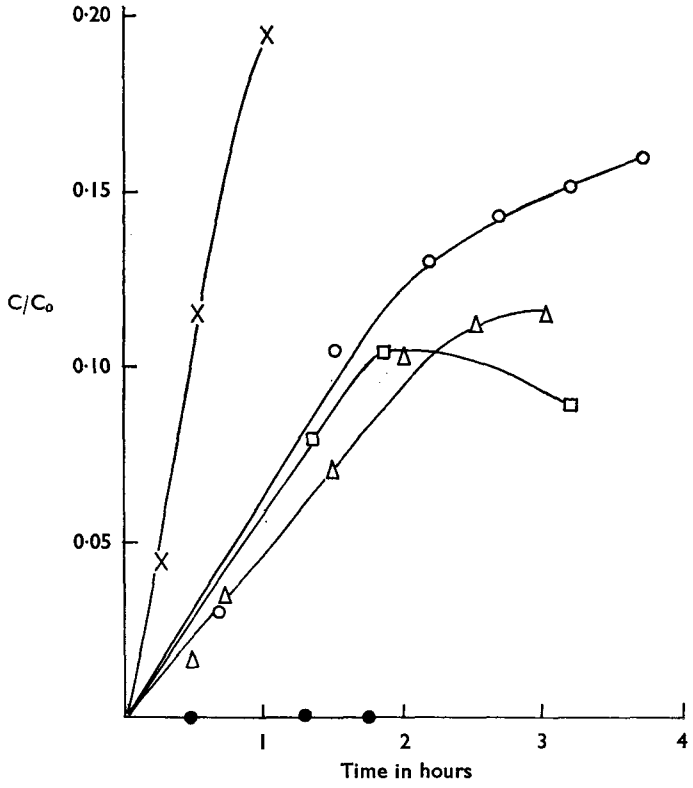

FIG. 2. Diffusion of 10^{-3}N $CaCl_2$. A plot of change in conductivity C, relative to initial conductivity of salt solution, C_0, against the time in hours, at a fixed height above the boundary. X, into water; O, into 2 per cent lec. 0·4 per cent lyso.; △ into 2 per cent lec.; □ into 5 per cent lec.; ● into 10 per cent lec.

phosphatide sol, the sol normally being the liquid below the boundary. Only 1 ml. of sol was required.

Sharp boundaries were formed by causing the sol and solution to flow off together through the exit tube in the left hand arm of the cell. Diffusion of salt into the sol was followed by measuring the conductivity between two platinum electrodes fixed 2 mm. above the boundary. Analar salts and demineralised water were used. The phosphatides were prepared as already described[3,4]. The sols were completely stripped of small electrolytes by treating them with mixed ion exchange resins; this

reduced their conductivity to an extremely low figure which remained unchanged for several days.

Much work has been devoted to attempting to obtain a system in which no diffusion of salt into the sol occurred, that is to prepare a sol impermeable to calcium salts. Since it had been found that 0·5 per cent lecithin sols gave films of measurable strength, much of the earlier work was done with dilute sols of concentration up to 2 per cent. In a number of experiments some retardation of calcium chloride diffusion was noted (see Fig. 2). The influence of additional components such as lysolecithin, cholesterol and plasma albumen was examined but no particularly significant effects were found.

A more concentrated sol containing 5 per cent each of lecithin and serum albumen did show some limited permeability effects. The salt

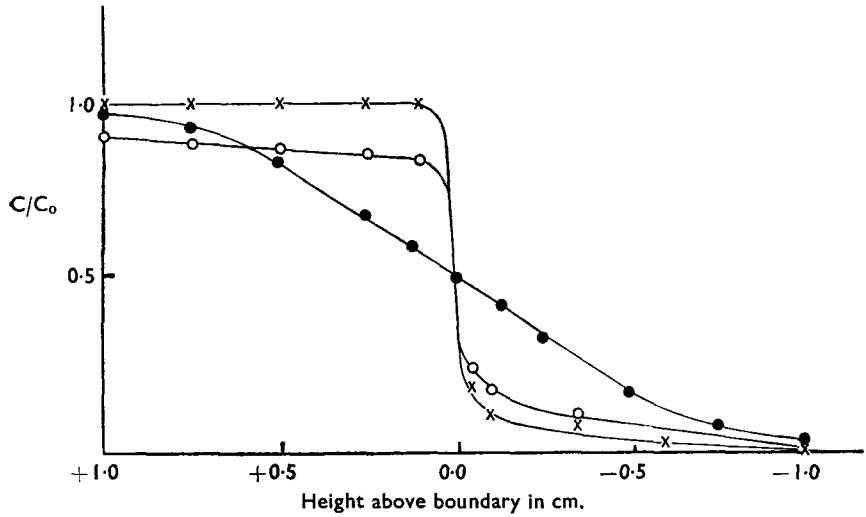

FIG. 3. Plots of conductivity relative to initial conductivity of salt solution C_0 against height above the boundary for 10^{-3} N $CaCl_2$ at 20 hours. ● into water; ○, into 5 per cent sol; X, into 10 per cent sol.

solution conductivity dropped at first, became constant and then began to increase slowly. This indicated that diffusion into the sol had stopped, presumably as a result of the slow formation of an impermeable film at the boundary. A similar effect (see Fig. 2) was obtained with a 5 per cent lecithin sol without albumen.

When these sol-solution systems were left overnight, the boundary remained extremely sharp. Mixed phosphatide sols containing more than 1 part of lysolecithin to 10 of lecithin showed some retardation of diffusion of calcium chloride (Fig. 2) and the boundary remained in its initial position at the exit tube; diffusion of salt into the sol could be seen as an opacity spreading down into the sol. Pure lecithin sols and sols containing a lower proportion of lysolecithin gave an apparent diffusion of salt, since the conductivity readings decreased with time.

However, overnight the boundary fell and it seems that in these systems a relatively impermeable film is formed. Osmotic effects then cause the sol to exude water so that the boundary falls and the salt solution above the boundary is diluted.

An interesting effect was noted with more concentrated lecithin sols (above 5 per cent). On standing alone overnight, a separation into two liquid layers (co-acervation), occurred. The upper layer was clear and the lower layer as a thick liquid containing most of the lecithin.

When the concentration of lecithin in the diffusion cell was increased to 10 per cent, the effect which had been sought throughout this work, was found. No change of conductivity of the salt solution in contact with this sol was observed. To confirm the impermeability of the sol to calcium chloride, a technique for scanning the column in the left hand capillary of the cell, was developed. The solution: sol boundary was formed and left for 20 hours, the liquid in the left hand capillary was then carefully moved up and down the tube and conductivity readings were taken in various positions relative to the sharp boundary. These measurements confirmed the relative impermeability of the 10 per cent sol. Figure 3 shows the results of scanning studies with 5 and 10 per cent sols together with a curve for the diffusion of calcium chloride into water after 20 hours. Immediately below the boundary, the lecithin sol had shrunk away from the sides of the tube slightly leaving a thin layer of clear liquid around it, this is the reason for the measurable conductivity just below the boundary.

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After the Author had presented the paper there was a DISCUSSION.