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A DEMONSTRATION OF ION-EXCHANGE PHENOMENA IN PHOSPHOLIPID MONO-MOLECULAR FILMS

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SINCE Rb^+ is a substitute for K^+ and Li^+ is a substiute for Na^+ in axonal potentials[1–3], the size and chargetof an ion have been thought by some to determine its pass age through the cell membrane[4]. The ionic radius of Cs^+ is 0·21 Å greater than the ionic radius of Rb^+ and is 1·09 Å greater than the ionic radius of Li^+ (ref. 5). One might expect, therefore, that Cs^+ would substitute for K^+. Experiments indicate, however, that Cs^+ acts neither like K^+ nor like Na^+ (refs. 2, 6). It has also been proposed that La^{+++} can displace Ca^{++} on the outer surface of the cell, since La^{+++} reversibly blocks the excitability of the axon and, when it is used as a stain in electron microscopy, the gap substance between myelinated axons appears densely stained[7,8]. In contrast with the large number of demonstrations of ion effects on the axon membrane, there have been relatively few experiments on the chemistry of these phenomena. The use of monomolecular films as structural models of the cell membrane[9] provides a system the properties of which could, by analogy, give insight into this matter.

Earlier experiments[9,10] indicate that among the molecular components of the cell membrane, phosphatidylserine may provide negatively charged sites for cation exchange. In the present work, therefore, emphasis was placed on phosphatidylserine films. Silicic acid chromatography was used to isolate this phospholipid from 'animal cephalin' (Nutritional Biochemical Co.) and its purity was tested by ascending paper chromatography[9].

The uptake of Ca^{++} from a hypophase containing 0·1 mmole calcium chloride at *p*H 6·00 ± 0·25 was measured by counting the number of β particles emanating from the surface of a hypophase containing calcium-45 before and after spreading of a phospholipid monolayer. An increase

in count was interpreted to indicate adsorption of Ca^{++} to the film. The technique has been described in detail elsewhere[9].

The experiments of displacing Ca^{++} from phosphatidylserine monolayers by Li^+, Rb^+ and Cs^+ were designed to show whether these monolayers would discriminate between Li^+, Rb^+ and Cs^+. The amount of calcium-45 adsorbed by the film in the presence of different concentrations of lithium chloride, rubidium chloride and caesium chloride in the hypophase was taken as a measure of the efficiency of displacement of Ca^{++} by Li^+, Rb^+ and Cs^+.

Fig. 1 shows that Li^+, Rb^+ and Cs^+ each displace the same number of calcium ions from the polar groups of the phosphatidylserine monolayer at any given concentration. This experiment substantiates previous findings[9] with Na^+ and K^+ and demonstrates that there is no discrimination among these monovalent cations. Also, it demonstrates that Ca^{++} adsorption depends on competing cation concentration.

The polar head of a phospholipid molecule would have a variable net ionic effect as determined by polar group configuration. It was thought that contaminating the monolayer with an interacting molecule such as cholesterol[11,12] would alter the polar group configuration so as to modify the position of the different ionogenic groups of the phospholipid molecule as previously postulated[13]. If this were so, the contaminated monolayer would show different ion association properties and, perhaps, selectivity. Fig. 1 shows that addition of cholesterol (Sigma Chemical Co.) does not modify the result obtained with phosphatidylserine alone. For example, the number of calcium ions per phosphatidylserine molecule is $0{\cdot}23 \pm 0{\cdot}04$ for phosphatidylserine monolayers and is $0{\cdot}19 \pm 0{\cdot}05$ for 50 per cent phosphatidylserine; 50 per cent choles-

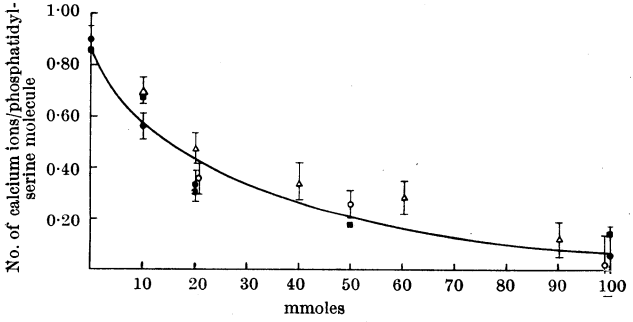

Fig. 1. Calcium displacement as a function of hypophase competing cation concentration. The vertical axis represents the number of calcium ions per phosphatidylserine molecule. The horizontal axis represents the concentration of competing counter ion in the hypophase. Each point represents the average of 3–8 experiments and the vertical bars represent twice the standard error. Area per molecule about 58 Å^2. Hypophase and monolayer. ●, CsCl, 0·1 mmole $CaCl_2$, PS; ○, LiCl, 0·1 mmole $CaCl_2$, PS; △, RbCl, 0·1 mmole $CaCl_2$, PS; ■. CsCl, 0·1 mmole $CaCl_2$, PS/C. PS, Phosphatidylserine; C, cholesterol

terol monolayers when the hypophase contained 50 mmole caesium chloride.

Thus, it seems to have been established that the adsorption of Ca^{++} by phsophatidylserine monolayer is equally prevented by equal concentrations of any of the following cations: Li^+, Na^+ (ref. 9), K^+ (ref. 9), Rb^+ and Cs^+. There are, however, some uncertainties about whether or not ion exchange occurs. Furthermore, no information is available about the time-course of this ion exchange.

The adsorption of Pm^{+++} (promethium) from a promethium chloride ($PmCl_3$) hypophase at *p*H 6·00 ± 0·25 was measured to determine whether or not ion exchange really occurs between the polar groups and the hypophase and to compare binding efficiency of Ca^{++} and La^{++} by measuring promethium-147 adsorption at different concentrations of calcium chloride or lanthanum chloride ($LaCl_3$).

Fig. 2 shows the time-course of the promethium-147 adsorption when the hypophase contains calcium chloride or lanthanum chloride. It can be seen that time is needed to reach the steady adsorption. Since phsophatidylserine is in its hydrogen form at the beginning of the experiment (as determined by flame photometry), the increase in counts measures the exchange of hydrogen (or Ca^{++} or La^{+++} also present in the hypophase) by Pm^{+++}. It can also be seen that about 500 times more Ca^{++} than La^{+++} is needed to produce the same equilibrium adsorption, starting from the same promethium chloride hypophase. From this experiment it can be concluded that (*a*) ion exchange actually occurs between the polar groups of the

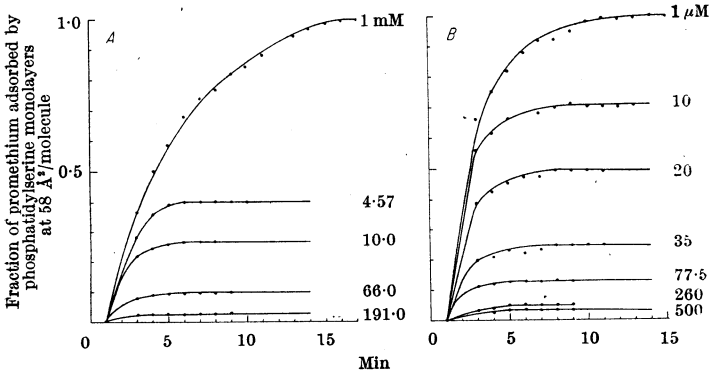

Fig. 2. Vertical axis represents the fraction of promethium adsorbed by phosphatidylserine monolayers, that is, adsorption of promethium when the hypophase contains a given concentration of calcium chloride (Fig. 2*A*) or lanthanum chloride (Fig. 2*B*) divided by the adsorption of promethium when the hypophase contained only 1 mmole lanthanum chloride. Each curve represents one experiment. Horizontal axis represents time in min starting with the solution without the monolayer. The film was spread at time 1 min and the maximum increase in count due to the film was 20,000 c.p.m. *A*, Hypophase, 1 μmole lanthanum chloride and calcium chloride; *B*, hypophase, lanthanum chloride

phospholipid mono-molecular film and the hypophase and (*b*) that La^{+++} is a strong competitor for the Ca^{++} binding sites. Therefore, the notion that La^{+++} displaces Ca^{++} from binding sites at cell surfaces seems to be well supported and could explain why La^{+++} reversibly blocks the excitable membrane and why the gap substance between myelinated axons appears densely stained when La^{+++} is used as stain in electron microscopy[7,8]. Finally, the idea of an ionic exchanger polar-group phase associated to phosphatidylserine mono-molecular films[9] seems to be supported and is consistent with both the molecular model for the cell membrane[14] and ion-exchange properties postulated for cell surfaces[15,16].

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[1] Araki, T., Ito, M., Oscarsson, P. G., and Oschima, T., *Nature*, **196**, 1319 (1962).
[2] Baker, P. F., Hodgkin, A. L., and Shaw, T. I., *J. Physiol.*, **164**, 355 (1962).
[3] Chandler, W. K., and Meves, H. (in preparation).
[4] Mullins, L. J., *J. Gen. Physiol.*, **43**, Suppl. 1, 105 (1960).
[5] Robinson, R. A., and Stokes, R. H., in *Electrolyte Solutions*, 450 (Butterworths Scientific Publications, 1955).
[6] Pickard, W. F., Lettvin, J. Y., Moore, J. W., Takata, M., Pooler, J., and Bernstein, T., *Proc. U.S. Nat. Acad. Sci.*, **52**, 1177 (1964).
[7] Lettvin, J. Y., Pickard, W. F., Moore, J. W., and Takata, M., *Quart. Prog. Rep., R. L. E., M.I.T.*, **75**, 159 (1964).
[8] Doggenweiler, C. F., and Frenk, S., *Proc. U.S. Nat. Acad. Sci.*, **53**, 425 (1965).
[9] Rojas, E., and Tobias, J. M., *Biochim. Biophys. Acta*, **94**, 394 (1965).
[10] Nash, H. A., and Tobias, J. M., *Proc. U.S. Nat. Acad. Sci.*, **51**, 476 (1964).
[11] Leathes, L. B., *Lancet*, **208**, 853, 957, 1019 (1925).
[12] De Bernard, L., *Bull. Soc. Chim. Biol.*, **40**, 161 (1956).
[13] Dervician, D. G., *Prog. Biophys. Mol. Biol.*, **14**, 265 (1964).
[14] Danielli, J. F., in Davson, H., and Danielli, J. F., *Permeability of Natural Membranes* (Cambridge University Press, London, 1952).
[15] Teorell, T., *Prog. Biophys. Chem.*, **3**, 305 (1953).
[16] Tobias, J. M., *Nature*, **203**, 13 (1964).

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