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Discrimination of Na^+ and K^+ by monolayers of lipids from epithelial cells

This is a study of the behaviour of monolayers formed with lipids extracted from epithelial cells. Several lines of evidence have indicated that lipids play a central role in selective ionic movement across epithelial membranes:

(1) Studies of the surface properties of phospholipid monolayers on subphases containing pure water[1] or solutions of different ions[2,3] demonstrate that the presence of ions in the subphase increases the area per molecule of lipids (L-α-dipalmitoyl lecithin), the surface potential and the surface dipole moment per molecule in the order $Na^+ > K^+ \approx Li^+ > H_2O$; the surface viscosity and the energy of activation of viscous flow decrease in the order $H_2O > Na^+ > K^+ > Li^+$.

(2) Studies of the process of Na^+ movement across epithelial membranes indicate that Na^+ transport involves a specific interaction with sites having a high $Na^+ > K^+$ selectivity[4–7] and that these sites are constituted by the polar groups of the lipids of the outer leaflet of the plasma membrane of the epithelial cells[8].

(3) Ionic movements across epithelial membranes are associated with their lipid composition[9].

This indicates the necessity of studying whether the lipids extracted from the epithelial cells, when they are distributed in a leaflet-like arrangement, can react distinctively in the presence of different cations, as single lipid species do[2,10].

Abdominal skins of *Leptodactylus ocellatus* were dissected, minced and collected in an erlenmeyer flask containing a solution (Ringer A) composed of 115 mM NaCl, 2 mM KCl, 200 mg/l EDTA (sodium salt), 1 g/l glucose, 0.21 mM NaH_2PO_4, 0.58 mM Na_2HPO_4. The pH was 7.2. 300 mg/100 ml trypsin were added, and the mixture was incubated and stirred at 37° during 20 min. After this period the supernatant containing free cells was extracted and centrifuged 10 min at 800 × *g*. The cells were resuspended in a solution (Ringer B) containing 115 mM NaCl, 2.4 mM $KHCO_3$, 1 mM $CaCl_2$, 1 g/l glucose and 6 g/l bovine serum albumin. The whole process was repeated 5 times. The dismemberment of the epithelium was followed by staining the isolated cells with crystal violet and observing the preparation under a microscope (L. GERSCHENSON AND D. CASANELLO, private communication). Once the activity of the trypsin was inhibited by the addition of Ca^{2+} and by lowering the temperature to the room level, the cells were centrifuged again and transferred to a tissue homogenizer made out of Pyrex. Ringer B was added, and the cells were then disrupted with a Teflon pestle, this was followed by a centrifugation at 1900 × *g* during 10 min. Although this pellet is not composed only of plasma membrane, the lipids it contains come mainly from this membrane and the nuclear one as well. The supernatant was discarded and the lipid component of the rest was extracted with chloroform–methanol (2:1, v/v) according to the method of FOLCH[11]. Thin-layer chromatography of this extract indicated the presence of phosphatidyl ethanolamine, cardiolipin, phosphatidyl choline (the most abundant phospholipid), cholesterol and glycerides. Lysolipids were present in small quantities. The phosphorous content as analysed by the method of BERENBLUM AND CHAIN[12] was 0.83 %. The lipid monolayer at the air–water interface was obtained by spreading the lipid extract dissolved in a mixture of absolute ethanol and purified light petroleum (b.p. 35–65°) (2:8, v/v). The specifications

of the reagent grade inorganic chemical and the water used in the preparation of the subphase solutions, as well as the criterion of surface purity for the air–saline solutions interfaces, have already been described[2]. Surface pressure (π–area) and surface potential (ΔV–area) curves were obtained at a constant compression rate with an automatic recording surface balance provided with an ^{241}Am air electrode; the temperature of the trough was maintained at 21° (± 0.2°) by circulating water from a constant temperature bath.

Fig. 1 shows the π–A and ΔV–A curves of the lipids spread on the following subphases: water, 150 mM NaCl, 150 mM KCl, 1 mM $CaCl_2$, 150 mM NaCl *plus* 1 mM $CaCl_2$ and 150 mM KCl *plus* 1 mM $CaCl_2$. Each curve represents the average of at least four experiments. All curves show a phase transition similar to the one observed with monolayers of synthetic dipalmitoyl lecithin[1,2]. When the subphase contains cations, the curves also exhibit a different expansion effect, as compared with those obtained at the air–pure water interface. The difference between the expansion effect of Na^+ and K^+ is eliminated by the presence of 1 mM Ca^{2+} in the subphase in such a way that the Na^+ *plus* Ca^{2+}, and K^+ *plus* Ca^{2+} compression curves coincide within the experimental error.

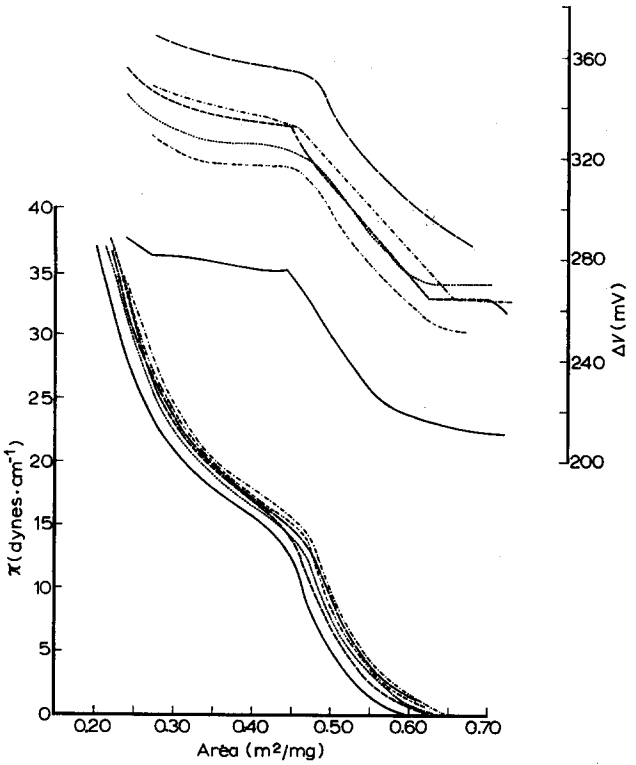

Fig. 1. Surface pressure (π) and surface potential (ΔV) as a function of area (A) for lipids extracted from epithelial cells of the frog skin at the air–water (———); air–NaCl (-------); air–KCl (-·—·-); air–$CaCl_2$ (·····); air–K^+ *plus* Ca^{2+} (—··—); and air–Na^+ *plus* Ca^{2+} (— — —) subphases. Temp., 21 ± 0.2°. The experimental errors, not included in the curve to avoid crowding, are: voltage curves: <8 mV; π–A curves: <0.0050 m^2/mg.

The surface potential increases when cations are present in the subphase as compared with the ΔV–A curves corresponding to the air–pure water interface. This effect is similar to that described for synthetic dipalmitoyl lecithin. It can be seen that the ΔV–A curves for Na^+, K^+ or Ca^{2+} coincide within the experimental error, but the presence of 1 mM Ca^{2+} produces a neat differentiation of the ΔV–A curves for the Na^+ *plus* Ca^{2+} and K^+ *plus* Ca^{2+} subphases.

In systems composed of pure lipids that permit a thorough analysis of the behaviour of the individual molecules in the monolayer, the change in the slope observed between 12 and 16 dynes is associated with the arrangement of the molecules in two-dimensional crystal-like structure, while the change in ΔV–A is thought to reflect the position of the ester links and the orientation of the polar groups[1–3,13]. Both of these characteristics of the monolayers are affected by the nature of ions in the subphase. Drawing an analogy one may conclude that the lipids extracted from the epithelial cells can also arrange themselves in ordered structures, and their polar heads react by varying their position as a function of the ion present in the subphase. It is not surprising that such an heterogeneous mixture of lipids could give rise to an ordered array, in view of the fact that LUZZATTI[14] has shown that lipid mixtures can exhibit phases as ordered as those observed with pure lipids and can also change from one to another, the main difference from pure lipids being a graded transition from one phase to the other instead of a sharp one.

The evidence that the lipids extracted from epithelial cells can react differently with the different ionic environments is very suggestive in view of the fact mentioned in the introduction that epithelial cells react very specifically with certain ions and that this property is associated with the lipid component of their component of the membrane. It is premature, though, to assign any functional implication to the phenomena described here. Even when the lipid extract used is likely to contain a large fraction of lipids from the plasma membrane, it by no means represents only these lipids. The present results, though, encourage a thorough study in which not a total extract but the lipids belonging to the different membranous structures are used.

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