between two aqueous phases as well as for studies of the interaction of adsorbates with such films. Tocopherol membranes will, therefore, help define the forces in the more complex lipid–lipid or lipid–protein interaction of biological membranes.

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1 P. MUELLER, D. O. RUDIN, H. T. TIEN AND W. C. WESCOTT, *J. Phys. Chem.*, 67 (1963) 534.
2 W. D. SEUFERT, *Nature*, 207 (1965) 174.
3 P. LÄUGER, W. LESSLAUER, E. MARTI AND J. RICHTER, *Biochim. Biophys. Acta*, 135 (1967) 20.
4 A. M. LIQUORI AND C. BOTRÉ, *J. Phys. Chem.*, 71 (1967) 3765.
5 D. O. SHAH AND J. H. SCHULMAN, *J. Lipid Res.*, 8 (1967) 215.
6 M. M. STANDISH AND B. A. PETHICA, *Biochim. Biophys. Acta*, 144 (1967) 659.
7 A. FINKELSTEIN AND A. CASS, *Nature*, 216 (1967) 717.
8 M. TAKAGI, K. AZUMI AND U. KISHIMOTO, *Ann. Rept. Biol. Osaka Univ.*, 13 (1965) 107.
9 H. T. TIEN AND S. CARBONE, *Nature*, 212 (1966) 718.
10 H. T. TIEN AND E. A. DAVIDOWICZ, *J. Colloid Interface Sci.*, 22 (1966) 438.

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The effect of potassium permanganate on lecithin and cholesterol monolayers*

Salts of heavy metals have been used extensively as stains in electron microscopy of cell organelles and membranes. However, little is known about the interaction of these heavy metal ions with lipids and proteins in membranes. Therefore, we have undertaken a study on the interaction of heavy metal ions with phospholipid and cholesterol monolayers as a model lipid membrane. The interaction of OsO_4 and uranyl acetate with lecithin and cholesterol monolayers and liquid crystals has been reported previously[1–3].

Chromatographically pure L-α-dipalmitoyl lecithin was purchased from Mann Research Laboratory (New York, N.Y.); egg lecithin was supplied by the Sylvana Chemical Co. (Orange, N.J.). Both lecithins were chromatographically pure and gave single spots on a thin layer chromatographic plate with a chloroform–methanol–water (60:35:5, by vol.) solvent system. The fatty acid composition of egg lecithin, which contains approximately equal amounts of saturated and unsaturated fatty acids, has been reported previously[4]. High purity cholesterol was supplied by Applied

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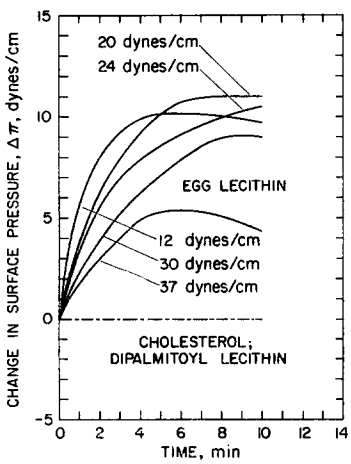
Science Laboratories (State College, Pa.). Inorganic chemicals of reagent grade and twice distilled water were used in all experiments.

Surface pressure and surface potential were measured by methods described previously[5]. Surface measurements were taken on subsolutions of 0.02 M NaCl at pH 6.0 and 25°. Monolayers were first compressed to a desired surface pressure and then 1 ml of $KMnO_4$ solution (concn. 12 mg/ml) was injected into the subsolution (400 ml). While the subsolution was agitated by a magnetic stirrer, similar to that employed in the enzymic hydrolysis of lecithin monolayers[4], changes in surface pressure and potential were recorded at time intervals of 1 or 1.5 min. All films were studied in duplicate and the results were not significantly different. However, without the stirring device the reproducibility was poor.

Fig. 1 shows the changes in surface pressure of cholesterol, dipalmitoyl and egg lecithin monolayers after injection of $KMnO_4$ in the subsolution. It is evident that cholesterol and dipalmitoyl lecithin monolayers are not influenced by $KMnO_4$ at any surface pressure, whereas egg lecithin monolayers show an increase in the surface pressure. However, after an initial rapid increase, the surface pressure showed a gradual decrease. The increase in surface pressure is due to interaction of the double bonds of fatty acyl chains with $KMnO_4$ in the subsolution. The rate of increase of surface pressure depends upon the initial state of compression of egg lecithin monolayers. A greater rate of change of surface pressure at low initial surface pressures suggests that the double bonds in the fatty acyl chains are more easily pulled towards the aqueous phase at low surface pressures than at high surface pressures. It is interesting to note that even at a surface pressure of 37 dynes/cm, the reaction between the fatty acyl chains of egg lecithin and $KMnO_4$ occurs. HUGHES AND RIDEAL[6] have similarly shown that the rate of oxidation of oleic acid monolayers by $KMnO_4$ decreases with increasing surface pressure of the monolayers.



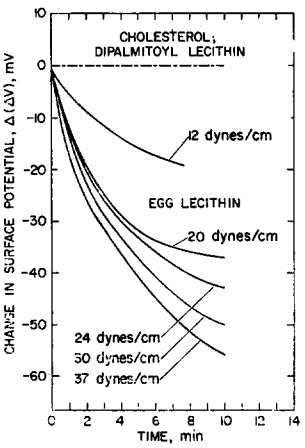


Fig. 1. Effect of $KMnO_4$ on the surface pressure of egg lecithin monolayers initially compressed to different surface pressures. For cholesterol and dipalmitoyl lecithin monolayers, there were no changes in surface pressure when $KMnO_4$ was injected under the monolayers maintained at different surface pressures.

Fig. 2. Effect of $KMnO_4$ on surface potentials of egg lecithin monolayers initially compressed to different surface pressures. For cholesterol and dipalmitoyl lecithin monolayers there were no changes in the surface potential when $KMnO_4$ was injected under the monolayers maintained at different surface pressures. (12 mg $KMnO_4$ in 400 ml of 0.02 M NaCl subsolution at pH 6.0.)

MARSDEN AND RIDEAL[7] have further shown that at high surface pressures the rate of oxidation by $KMnO_4$ is greater for fatty acid chains having *cis-* rather than *trans-* double bonds. The mechanism of reaction between $KMnO_4$ and a double bond is not yet well understood. However, GILBY AND ALEXANDER[8] have attributed the initial increases in surface pressure to the formation of the dihydroxy acid[9]. The hydroxy groups added on the double bond are pulled towards the aqueous phase and thereby increase the surface pressure. Subsequently, further oxidation occurs at this point with ultimate fission and formation of two carboxyl groups. The resulting short-chain molecules are soluble in the subsolution and hence leave the monolayers causing a decrease in the surface pressure[8]. This explains why there is an initial increase and subsequent slow decrease in the surface pressure of egg lecithin monolayers. The results shown in Fig. 1 also suggest that the rate of dissolution of short-chain molecules from oxidized monolayers depends upon the initial state of compression of the monolayers.

Fig. 2 shows the corresponding changes in surface potentials of cholesterol, dipalmitoyl and egg lecithin monolayers when $KMnO_4$ was injected in the subsolution. Cholesterol and dipalmitoyl lecithin monolayers are not influenced by $KMnO_4$, whereas egg lecithin monolayers exhibit a decrease in surface potentials. In contrast to surface pressure, the rate of change of potential increases with the surface pressure. This can be explained as follows: The increase in the surface pressure is presumably due to fatty acyl chains being pulled down at the air–water interface upon oxidation, whereas the decrease in the surface potential is mainly due to oxidation of the double bonds. The fall of surface potentials at high surface pressures, therefore, suggests that the fatty acyl chains are oxidized at high surface pressure but not pulled down at the interface to the same extent as that at low surface pressures. The greater rate of change of surface potential at high surface pressures is probably the result of a greater surface concentration of molecules in the monolayer (*i.e.* the number of double bonds per cm^2 is greater at high surface pressures). We should point out that at pH's near neutrality, the double bonds in the fatty acyl chains are oxidized by $KMnO_4$, whereas those in cholesterol molecules are not. However, at pH 2–3, the double bond of cholesterol is oxidized by $KMnO_4$ resulting in an increase of 170–175 mV in the surface potential of cholesterol monolayers.

A comparison of the previous works on the effect of OsO_4 and uranyl acetate on lecithin and cholesterol monolayers[1–3] with the present results indicates that both $KMnO_4$ and OsO_4 react with the double bonds of fatty acyl chains but not with phosphorylcholine groups in lecithin monolayers, since they do not influence the surface pressure or potential of dipalmitoyl lecithin monolayers. If there had been ionic or ion dipole interaction between phosphorylcholine group and $KMnO_4$ or OsO_4, it would have changed the surface potential of dipalmitoyl lecithin monolayers. The presence of double bonds is necessary for the interaction of OsO_4 or $KMnO_4$ with lecithin monolayers. In contrast, uranyl ions react with the phosphate groups of both saturated or unsaturated lecithins, increase the surface potential by 200–250 mV and tend to solidify lecithin monolayers[2].

The interaction of OsO_4 with the double bond of cholesterol decreases the surface potential by 115 mV (ref. 1), whereas $KMnO_4$ and uranyl acetate do not influence the surface potential of cholesterol monolayers[2]. However, at acidic pH, $KMnO_4$ reacts with cholesterol monolayers and increases the surface potential by 170–175 mV.

These results indicate that the mechanism of interaction of $KMnO_4$ with cholesterol monolayers is different from that of OsO_4, because these metal salts have opposite effects on surface potentials. The effects of $KMnO_4$ on various lipid monolayers have been reviewed recently by SHAH[10]. MORETZ *et al.*[11] have shown, using the small-angle X-ray diffraction method, that both OsO_4 and $KMnO_4$ caused a significant rearrangement of molecules in the myelin membrane. The changes in surface pressures and potentials of lecithin monolayers reported here support the view that $KMnO_4$ could significantly alter the arrangement of lipid molecules in the membrane due to its interaction with the double bonds in the fatty acyl chains of phospholipids.

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1 K. D. DREHER, J. H. SCHULMAN, O. R. ANDERSON AND O. A. ROELS, *J. Ultrastruct. Res.*, 19 (1967) 586.
2 D. O. SHAH, *J. Colloid Interface Sci.*, 29 (1969) 210.
3 O. R. ANDERSON, O. A. ROELS, K. D. DREHER AND J. H. SCHULMAN, *J. Ultrastruct. Res.*, 19 (1967) 600.
4 D. O. SHAH AND J. H. SCHULMAN, *J. Colloid Interface Sci.*, 25 (1967) 107.
5 D. O. SHAH AND J. H. SCHULMAN, *J. Lipid Res.*, 6 (1965) 341.
6 A. H. HUGHES AND E. K. RIDEAL, *Proc. Roy. Soc. London, Ser. A*, 140 (1933) 253.
7 J. MARSDEN AND E. K. RIDEAL, *J. Chem. Soc.*, (1938) 1163.
8 A. R. GILBY AND A. E. ALEXANDER, *Australian J. Chem.*, 9 (1956) 347.
9 T. P. HILDITCH, *J. Chem. Soc.*, (1926) 1828.
10 D. O. SHAH, in R. PAOLETTI AND D. KRITCHEVSKY, *Advan. Lipid Res.*, (1970) in the press.
11 R. C. MORETZ, C. K. AKERS AND D. F. PARSONS, *Biochim. Biophys. Acta*, 193 (1969) 1.

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