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SMALL MOLECULE–LIPID MEMBRANE INTERACTIONS AND THE PUNCTURING THEORY OF OLFACTION

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SUMMARY

The effect of aliphatic alcohols and other small molecules on the resistance of lecithin bilayers has been examined. It is found that short-chain alcohols decrease the bilayer resistance, while above C_8 the bilayer resistance is increased. It is proposed that these effects are due to changes in the fluidity of the lipid hydrocarbon chains. In addition to the alcohols, a variety of other molecules induce small conductance changes in the bilayer. The relevance of these findings to the puncturing theory of olfaction and other small molecule-membrane interactions is discussed.

INTRODUCTION

The molecular basis of the olfactory transduction mechanism is not known. Of the many theories of olfaction which have been proposed, that of DAVIS and co-worker[1–3] is noteworthy in that it provides both an explicit mechanism for the excitation process and a quantitative estimate of olfactory thresholds. According to DAVIES AND TAYLOR, the odorant molecule is adsorbed onto a lipid membrane. The dynamic processes of adsorption and desorption produce a temporary "puncturing" of the membrane. The receptor potential is initiated by the resulting localised increase in the permeability of the membrane to Na^+ and K^+.

Experimental support for this theory has come from studies of the acceleration by odorants of saponin-induced haemolysis[4]. The precise relationship between this somewhat complex system and the proposed olfactory mechanism is, however, by no means clear. The recent development of techniques for forming single lipid bilayers has enabled us to make a much more direct test of the mechanism of DAVIES AND TAYLOR.

Various studies have now clearly established that the lipid bilayer is virtually impermeable to small ions and hence has a very low electrical conductivity (see the reviews of BANGHAM[5], HENN AND THOMPSON[6] and MUELLER AND RUDIN[7]). Thus conductance measurements provide a very sensitive method of detecting any change in ionic permeability which might be produced by the adsorption of odorant molecules. In this paper we describe studies of the conductance of lipid bilayers in the

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presence of a variety of odorants; with particular emphasis on the homologous series of aliphatic alcohols.

MATERIALS AND METHODS

The bilayer apparatus was essentially similar to that described by MUELLER AND RUDIN[8]. The membrane was formed by the brush technique across a 1-mm diameter hole in a teflon pot. The membrane forming solution was 1% lecithin in *n*-decane and the aqueous solution was 0.1 M KCl in 0.1 M Tris buffer (pH 7.4).

A d.c. potential was applied to the bilayer and the current measured with a Keithley 610B Electrometer. The capacitance of the bilayer was measured by a bridge method at a frequency of 1000 cycles/sec using a Wayne Kerr B221A Universal Bridge. All measurements were made at room temperature.

The procedure for studying the effects of odorants on the conductance was as follows. After the bilayer had formed, the current was measured as a function of voltage over the range -50 to $+50$ mV. The odorant was then added to one compartment as a solution in either water or dioxan. Some of the alcohols were added directly. Sufficient buffer was then added to the other compartment to prevent bowing of the membrane. After allowing time for equilibrium to be reached a further current–voltage plot was obtained. Resistances were determined from the slope of the current–voltage plot.

The egg lecithin was prepared by a similar method to that of PANGBORN[9]. The lecithin was stored as a 10% solution in chloroform–methanol (20:1, by vol.), under N_2 at $-20°$. The lecithin was made up as a 1% solution in *n*-decane prior to use.

The odorant molecules were of the highest purity commercially available. No further purification was undertaken unless otherwise indicated. The alcohols were the purest grade from Fluka Chemical Co. and only freshly opened samples were used. The pure musk samples were generously donated by Dr. M. G. J. Beets of International Flavours and Fragrances, Hilversum. The KCl was spectroscopic grade, the *n*-decane was Koch-Light Puriss grade and the dioxan was purchased from Merck. Trizma grade Tris was supplied by Sigma.

RESULTS

Initially a large number of compounds were screened by obtaining current–voltage plots at one or two concentrations of the compound. Resistances were calculated from the slope of the current–voltage plots, which were ohmic in all cases. The intrinsic bilayer resistance was typically $1 \cdot 10^7$–$1 \cdot 10^8 \Omega \cdot cm^2$. The results obtained with all the compounds tested (except the alcohols) are summarised in Table I. Our experience of resistance measurements with lipid bilayers indicates that changes of less than 3 times are of doubtful significance and consequently only compounds which produced a change greater than this are regarded as having a definite effect. It may be seen from Table I that of the compounds listed, nearly half had no observable effect, while the remainder produced small resistance increases or decreases in approximately equal numbers. Only one compound, 2,4-dinitrophenol, produced a change greater than an order of magnitude, this result being in agreement with previous studies with this compound[10,11].

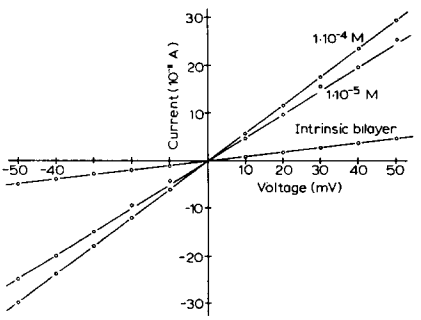
TABLE I

EFFECTS OF ODORANTS ON THE RESISTANCE OF LECITHIN BILAYERS

The odorants were added to the aqueous phase in the concentration range $1 \cdot 10^{-3}$–$1 \cdot 10^{-5}$ M or, when the solubility was lower than this, as saturated solutions. "No significant change" indicates that any observed resistance change was less than 3 times.

Compounds which produced no significant change in bilayer resistance	Musk ambrette, musk 781, astrotone, celestolide, musk 665, heptadecanolide, hexane, cyclohexane, benzene, coumarin, α-ionone
Compounds which increased bilayer resistance by up to 10 times	Musk ketone, musk xylol, galaxolide, musk 63, β-ionone, nitrobenzene, valeric acid, pentabromophenol
Compounds which increased bilayer resistance by more than 10 times	None
Compounds which decreased bilayer resistance by up to 10 times	3,5-Dibutyl acetophenone, musk IV, phenol, scatole, isoamyl acetate, dimethyl sulphide
Compounds which decreased bilayer resistance by more than 10 times	2,4-Dinitrophenol

After this initial survey, the series of aliphatic alcohols was examined in more detail. For each alcohol, the resistance change was measured as a function of concentration. Typical results are shown in Figs. 1 and 2. The results for each alcohol were qualitatively reproducible but showed considerable quantitative variation from one run to another.



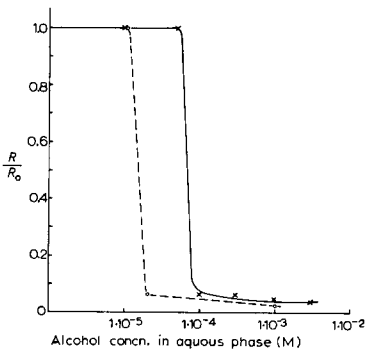


Fig. 1. Current–voltage plots for a lecithin bilayer with varying concentrations of *n*-pentanol added to the aqueous phase.

Fig. 2. Variation of bilayer resistance (R) with alcohol concentration. R_0 is intrinsic resistance of bilayer. ×———×, methanol; O– – – –O, pentanol.

All the alcohols gave ohmic current–voltage plots in the voltage range studied. The resistance changes were apparent on the earliest possible measurement after addition of the alcohol which was after about 1 min. The resistance at a particular alcohol concentration was constant with time (10–15 min). At the concentrations at

which the major resistance changes were observed, the alcohols had no noticeable adverse effect on the bilayer stability. However, further increase of alcohol concentration up to $1 \cdot 10^{-3}$–$1 \cdot 10^{-2}$ M resulted in rupture of the bilayer.

The results obtained for the series of alcohols are summarised in Fig. 3 where the maximum observed resistance change is plotted against chain length. Each point is the average of values obtained from several runs. The large uncertainty in each point in Fig. 3 prevents an accurate determination of the variation of resistance change with chain length to be made. Nevertheless, it is clear that a transition occurs around C_8, shorter chain alcohols producing a decrease in bilayer resistance and longer chain alcohols producing an increase. The concentration of alcohol required to produce the resistance change was generally in the order of 10^{-4} M and did not vary significantly with chain length.

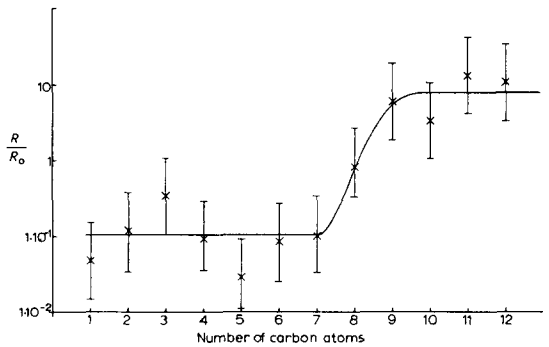


Fig. 3. Variation of maximum change of bilayer resistance (R) with alcohol chain length. R_0 is intrinsic bilayer resistance.

In addition to the above experiments a number of tests were carried out to ensure that the observed resistance changes were not artifacts. It was established that no significant change in bilayer resistance was produced by adding buffer alone, or by adding dioxan in the concentrations used in these experiments. In addition, in the case of the alcohols, the capacitance of the bilayer was found to remain constant during the titrations. Since the capacitance is directly proportional to the area of the bilayer, this established that the resistance changes were not due to variations in area.

DISCUSSION

We consider first the results we have obtained for the series of aliphatic alcohols. A change in resistance of the lipid bilayer may reflect either a change in the concentration of charge carriers in the bilayer or a change in their mobility. Following the treatment of PARSEGIAN[12], the concentration of ions in the bilayer is primarily dependent on the electrostatic energy required to place an ion in the low dielectric interior of the bilayer. This energy may be reduced by complexing the ion with a lipid soluble carrier or by the presence of high dielectric pores. A carrier mechanism would not be expected in the case of the alcohols. The pore mechanism also appears unlikely in view of the shape of the titration curves in Fig. 1. If alcohol molecules, either singly or in association, were inducing pores in the bilayer, one would expect

the number of pores and hence the conductance, to increase with alcohol concentration until the film becomes unstable. This is what is observed with the polyene antibiotics nystatin[13] and amphotericin[14], where there is good evidence for pore formation. In contrast, the bilayer conductance, in the presence of the lower alcohols, shows a rapid rise over a narrow concentration range followed by little or no variation with further increases in concentration. It should be pointed out that this effect cannot be explained in terms of a solubility limitation of the alcohol in the bilayer.

The shape of the titration curves in Fig. 2 does in fact suggest that a co-operative effect is taking place involving the bilayer overall rather than a local modification of bilayer structure. This is also suggested by the fact that the higher alcohols increase the resistance, since such an effect cannot be explained in terms of a localised change. Similar titration curves have also been observed with detergents[15,16] and with lysolecithin[17].

The biphasic form of the plot of resistance change (Fig. 3) against chain length indicates that the higher and lower alcohols interact differently with the bilayer. From their amphiphilic properties, we would expect the alcohols to be oriented in the bilayer with their hydroxyl group located in the polar region. The shorter chain alcohols will then have the effect of spacing out the lecithin molecules and reducing the hydrophobic interaction between the lecithin chains. This type of effect has previously been discussed by HAYDON AND TAYLOR[18] with reference to bilayer stability, and by LAWRENCE[19] with reference to hydrotropy. In the present instance, addition of high concentrations of alcohol does, in fact, lead to rupture of the bilayer. At lower alcohol concentrations the reduced lipid–chain interaction would be expected to result in increased fluidity of the lecithin chains. This effect will decrease as the chain length of the alcohol increases to fill the 'space' below the hydroxyl group. In fact no trend is observable in the molar concentration (in the aqueous phase) at which the major change in resistance occurs. However, since the longer chain alcohols will increasingly partition in favour of the lipid phase, the concentration of molecules in the bilayer needed to produce the resistance change is indeed increasing with chain length as expected.

It is difficult to predict the chain length at which the above effects would be expected to become negligible. There are, however, several observations which indicate a sharp change in the properties of alcohols and related amphiphiles at about C_8. Thus the alkyl trimethylammonium bromides are soaps down to C_8, below which properties change abruptly and the lower members are hydrotropes[19]. The interfacial tension between water and the normal alcohols themselves increases rapidly with chain length up to about C_7–C_8, after which there is little further change[20]. HAUSER AND DAWSON[21] found that the ability of aliphatic amines to displace Ca^{2+} from phosphatidylinositol films exhibited a discontinuity at about C_7. Finally the studies of BANGHAM *et al.*[22] are particularly relevant to the present work. These authors showed that the leakage of K^+ from liposomes was increased in the presence of the lower normal aliphatic alcohols. The effectiveness of the alcohols was found to decrease with increasing chain length. Their results suggest that the effect becomes negligibly small after C_8, although it is not stated whether higher alcohols were examined. These various effects may be regarded as reflecting the increase in hydrophobic interaction with increasing chain length.

The effect of butanol in increasing the K^+ permeability of liposomes was studied

in some detail by JOHNSON AND BANGHAM[23]. From the temperature dependence of the permeability they deduced that the main permeability barrier was located at the aqueous–lipid interface. As the enthalpy of activation for the K^+ permeability was unchanged by the presence of butanol, they deduced that the increase in permeability resulted principally from an entropy change. Their interpretation that the presence of butanol increases the freedom of movement of the lipid molecules is in general agreement with the present explanation of the resistance changes produced by the short-chain alcohols.

Above C_8, the alcohols actually increase the resistance of the lipid bilayer. This suggests that interaction between the alkyl chains of the higher alcohols and the lipid chains results in a reduction in the lipid chain motion. This is interesting in view of the studies of PHILLIPS *et al.*[24] with mixed lecithin systems. These authors found that the mixing of two components of different transition temperatures (T_c) changed the molecular motion of the higher melting component, but the lower melting component was unaffected. (The alcohols C_9–C_{12} all have melting points well above the T_c of egg lecithin[21].) However, R. M. WILLIAMS (unpublished data) has observed anomalous behaviour in the effect of stearyl alcohol on the lecithin–water system. The alcohol forms a complex with lecithin and the T_c of the mixed system is raised. This provides some support for the suggestion that the long-chain alcohols reduce the motion of the lipid chains.

The preceding discussion indicates that the fluidity of the hydrocarbon interior of the bilayer may be either increased or decreased by the presence of aliphatic alcohols, depending on their chain length. The simplest way in which this could cause corresponding resistance changes is by altering the mobility of the charge carriers in the bilayer. Alternatively the concentration of ions in the bilayer may be altered by a change in dielectric constant. In addition to the effects discussed above, a change in dielectric constant could also reflect a compositional change in the bilayer. Since no change in capacitance was observed in the presence of alcohols, any change in dielectric constant must be small. However, a change in dielectric constant of 1–3% would be sufficient to account for the observed conductance changes[12], a change of this magnitude being barely detectable in the capacitance measurements.

In addition to the aliphatic alcohols, Table I indicates that a variety of small molecules may induce resistance changes in lipid bilayers. The observed changes are, in general, small and may be either positive or negative. The only large effect was observed with 2,4-dinitrophenol, this result being in agreement with those of LIBERMAN *et al.*[10] and HOPFER *et al.*[11]. These previous studies were interpreted to indicate that 2,4-dinitrophenol probably acts as a carrier for protons across the bilayer. For the remainder of the molecules listed in Table I, it is clear that further data would be required in order to discuss profitably the mechanism of the resistance changes.

We now consider the implications of the present studies for the puncturing theory of olfaction. It is clear that the results obtained provide little support for such a mechanism. No correlation is apparent between the observed resistance changes and the olfactory thresholds of the molecules listed in Table I; or for that matter with their haemolytic accelerating power[4]. For example, the ionones which have very low olfactory thresholds and strongly accelerate saponin-induced haemolysis have no correspondingly pronounced effect on the bilayer resistance. In addition it appears that odorant molecules are as likely to increase as decrease the bilayer resistance.

In the case of the alcohols, it is more reasonable to suppose that the resistance changes result from alteration of the packing of the lipid chains rather than from a puncturing mechanism.

Furthermore, the observed resistance changes are, in general, small. It is well established that the intrinsic resistance of a lipid bilayer is several orders of magnitude above that of natural membranes[5–7]. Thus, although the structure of the olfactory membrane is not known, it is clear that any region of lipid bilayer will behave as an insulator, and that small changes in the resistance of this region are unlikely to have any effect on the overall electrical properties of the membrane.

Our results may also be relevant to other alcohol–membrane interactions such as are found in haemolysis, membrane stabilization, anaesthesia and trigeminal stimulation. In many instances, *e.g.* the inhibition of erythrocyte haemolysis[25], the log alcohol concentration required to produce a given effect is a linear function of the chain length throughout the series. However, this is not always the case; for example in the inhibition of reflex responses in tadpoles, a break occurs beginning with octanol[25]. Further, both the olfactory thresholds quoted by DAVIES AND TAYLOR[1] and the more recent measurements of LAFFORT[26] indicate that a minimum occurs at C_8. The relationship between these effects and the discontinuity observed at C_8 in the present bilayer studies is by no means clear. It could be that in these cases the alcohols are interacting with hydrophobic regions of the membrane in a similar manner to that suggested for the bilayer.

Finally, we consider the question of whether puncturing effects might be observed in bilayers of different composition from the egg lecithin–decane system used in the present experiments. This appears unlikely, since bilayer properties such as resistance, which are determined by the hydrocarbon interior, appear to vary little with lipid composition[5]. Another possible reason for the failure to observe a puncturing effect is that the bilayer is too fluid and any "pores" which are formed reseal too rapidly to allow any appreciable transfer of ions. Possibly this fluidity is reduced in the natural membrane by interaction with protein. Indeed, in the erythrocyte membrane there is evidence that this is the case[27]. However, any reduction in the fluidity of the membrane will make penetration by the odorant molecule more difficult. This effect will tend to compensate any increased sensitivity due to a greater probability of "pore" formation.

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REFERENCES

1 J. T. DAVIES AND F. H. TAYLOR, *Biol. Bull.*, 117 (1959) 222.
2 J. T. DAVIES, *Symp. Soc. Exptl. Biol.*, 16 (1962) 170.
3 J. T. DAVIES, *J. Colloid Interface Sci.*, 29 (1969) 296.
4 J. T. DAVIES AND F. H. TAYLOR, *Nature*, 174 (1954) 693.
5 A. D. BANGHAM, *Progr. Biophys. Mol. Biol.*, 18 (1968) 29.
6 F. A. HENN AND T. E. THOMPSON, *Ann. Rev. Biochem.*, 38 (1969) 241.
7 P. MUELLER AND D. O. RUDIN, *Current Topics Bioenergetics*, 3 (1969) 157.
8 P. MUELLER AND D. O. RUDIN, in H. PASSOW AND R. STÄMPFLI, *Laboratory Techniques in Membrane Biophysics*, Springer, Berlin, 1969, p. 141.

9 M. C. PANGBORN, *J. Biol. Chem.*, 188 (1951) 471.
10 Y. A. LIBERMAN, N. MOKHOVA, V. P. SKULACHEV AND V. P. TOPALY, *Biophysics USSR, Engl. Transl.*, 13 (1968) 188.
11 U. HOPFER, A. L. LEHNINGER AND T. E. THOMPSON, *Proc. Natl. Acad. Sci. U.S.*, 59 (1967) 484.
12 A. PARSEGIAN, *Nature*, 221 (1969) 844.
13 A. FINKELSTEIN AND A. CASS, *J. Gen. Physiol.*, 52 (1968) 145 S.
14 T. E. ANDREOLI, V. W. DENNIS AND A. M. WEIGL, *J. Gen. Physiol.*, 53 (1969) 133.
15 W. SEUFERT, *Nature*, 207 (1965) 174.
16 M. HASHIMUTO, *Bull. Chem. Soc. Japan*, 41 (1968) 2823.
17 H. VAN ZUTPHEN AND L. L. M. VAN DEENEN, *Chem. Phys. Lipids*, 1 (1967) 389.
18 D. A. HAYDON AND J. TAYLOR, *J. Theoret. Biol.*, 4 (1963) 281.
19 A. S. L. LAWRENCE, *Mol. Cryst.*, 7 (1969) 1.
20 C. O. TIMMONS AND W. A. ZISMAN, *J. Colloid Interface Sci.*, 28 (1968) 106.
21 H. HAUSER AND R. M. C. DAWSON, *Biochem. J.*, 109 (1968) 909.
22 A. D. BANGHAM, M. M. STANDISH AND N. MILLER, *Nature*, 208 (1965) 1295.
23 S. M. JOHNSON AND A. D. BANGHAM, *Biochim. Biophys. Acta*, 193 (1969) 92.
24 M. C. PHILLIPS, B. D. LADBROOKE AND D. CHAPMAN, *Biochim. Biophys. Acta*, 196 (1970) 35.
25 H. SCHNEIDER, *Biochim. Biophys. Acta*, 163 (1968) 451.
26 P. LAFFORT, *Rev. Laryngol.*, Suppl. to Oct. (1965) 860.
27 D. CHAPMAN, V. B. KAMAT, J. DE GIER AND S. A. PENKETT, *J. Mol. Biol.*, 31 (1968) 101.