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Interaction of hydrophobic organic compounds with mercury adsorbed dioleoylphosphatidylcholine monolayers

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The interaction of hydrophobic organic compounds with biological membranes is an important factor in their biological activity. A fundamental insight into the mechanisms can be obtained by examining the influence of these substances on model membrane systems. The effect of four groups of hydrophobic aromatic compounds on a mercury-adsorbed dioleoylphosphatidylcholine (DOPC) monolayer is described in this paper. The compounds studied were: (1) polynuclear aromatic hydrocarbons (PAH), (2) polychlorinated biphenyls (PCB), (3) neurotoxic pesticides, and (4) phenothiazines. The monolayer properties were measured using phase-sensitive a.c. voltammetry and cyclic voltammetry. The response of the monolayer to these compounds is recorded as a change in the form of the capacity-potential curve especially with respect to two capacity peaks which correspond to two well-defined phase transitions. Planar aromatic molecules cause a negative shift of the capacity peaks, however, as the molecule becomes more globular the response becomes less and the peaks become suppressed. It is shown that planar aromatic molecules exert their effect by penetrating the hydrocarbon region of the monolayer and that there is a molecular size cut-off for higher membered PAH molecules. In addition, the monolayer is not so sensitive to the bulky PCB which have a more disruptive effect on the phase transitions and thus on the mechanisms of self-assembly of the monolayer. A direct correlation is shown between the biological membrane activity of the phenothiazines and their effect on the monolayer at submicromolar levels. A molecular selectivity of phospholipid monolayers to these compounds is indicated which has implications for their effect on biological membranes.

Introduction

In spite of the fact that many toxic substances may ultimately exert their biological effect on proteins [1], the interaction of hydrophobic organic compounds with biological membranes will always represent a critical factor in their biological activity [2]. This applies equally to the diffusional entry of the compound into a cell where passive transport of the compound is of fundamental significance [3] or to the disruption of the structure of functional membranes inside the cell where the most important physiological mechanisms are located. Thus membrane-mediated processes such as photosynthesis, respiration and nerve impulse conduction are all dependent on the precise orientation of electron transferring enzymes and ion channels and any change in their position by a modification of the fundamental lipid backbone will alter the physiological process [4,5]. In addition, the important properties of lipid bilayers in membranes are their selective permeability, fluidity and ability to act as sensors of electric charge [6] and an effect by a foreign compound on these properties will have far reaching consequencies on membrane processes. Because of this it is mandatory to have an understanding of the mechanisms of interaction of hydrophobic compounds with biological membranes. Most notably it is necessary to understand in which way the compound most readily penetrates the membrane, what are the factors which hinder the penetration and how penetration mechanisms differ from one group of compounds

Abbreviations: DOPC, dioleoylphosphatidylcholine; PAH, polynuclear aromatic hydrocarbon; PCB, polychlorobiphenyl; *p,p'*-DDT, 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane; methoxychlor, 2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane; HMDE, hanging mercury drop electrode; a.c., alternating current; PZC, position of zero charge; K_{OW}, octanol-water partition coefficient; K_{CW}, chloroform-water partition coefficient; K_{LW}, adsorbed lipid layer-water partition coefficient.

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to another. It is of equal importance to know whether the compound radically alters the self-assembly forces which hold the membrane together in an organised state.

When commencing a study of this nature it is always useful to begin with a membrane model where all of the above mentioned effects can be accurately and readily measured. Interactions of hydrophobic compounds with lipid assemblies have been carried out previously with membrane models of vesicles (see, for example, Ref. 7) and lipid bilayers (see, for example, Ref. 4) but the system of a phospholipid monolayer adsorbed on to mercury has been introduced recently as an alternative experimental analogue of the biomembrane [8–10] and this model has already been shown to be very sensitive to polynuclear aromatic hydrocarbon penetration [11,12]. One of the main advantages of the system over other membrane models is the superior sensitivity of the electrochemical monitoring methods to perturbations in the monolayer structure enabling penetration effects due to organic compounds to be easily observed. The system also has an inherent reproducibility and stability relying on the self-assembly properties of the lipids on the adsorbing surface and thus experiments can be performed rapidly so that a large number of interactions can be examined consecutively. In this way it is ideal for measuring interactions between the monolayer and species in solution. In spite of the fact that this mercury-adsorbed lipid monolayer is a newly developed system, the processes of self-assembly of the lipids and the electrodic mechanisms in the electrode-adsorbed monolayer are now quite well understood [8–10,13,14]. As a result the mechanisms behind the penetration effects are more easily interpreted.

There is a great deal of interest in the biological activity of aromatic compounds both in respect of their toxicology [15] and also because several classes of aromatic compound are employed as highly specific drugs and pesticides [16]. However, to date there has been no attempt at a study of the membrane interacting properties of aromatic compounds to identify the general principles relating to the way in which the physicochemical characteristics of these molecules influence their membrane penetration. This paper thus reports studies on the interaction of groups of hydrophobic aromatic compounds with a mercury-adsorbed dioleoylphosphatidylcholine (DOPC) monolayer. The work has been carried out in order to answer the following questions:

(1) What are the molecular characteristics of hydrophobic aromatic compounds which are responsible for promoting interaction with mercury-adsorbed phospholipid monolayers and how does the sensitivity of the monolayers to these compounds depend on these properties?

(2) Can we deduce from this a mechanistic understanding of the nature of each interaction or group of interactions?

Four groups of compounds are considered in this study: (1) polynuclear aromatic hydrocarbons (PAH), (2) polychlorobiphenyls (PCB), (3) neurotoxic pesticides, and (4) phenothiazines. These groups of compounds have been chosen for study since; firstly, all groups of compounds have the ability to induce changes in biological systems and secondly, the compounds show variations in physicochemical properties such as aromaticity, planarity, hydrophobicity and size. In this way factors important in monolayer interaction can be identified. Throughout this study, particular attention has been paid to the relative sensitivity of the monolayer to these compounds since any compound will exert an effect on the monolayer at high enough concentration. The ultimate aim of this work is to advance the understanding of the effect of these substances on the lipid component of biological membranes.

Materials and Methods

Monolayers of dioleoylphosphatidylcholine (DOPC) are readily adsorbed on to mercury electrodes [8,9]. These are formed by depositing a monolayer on to a hanging mercury drop electrode (HMDE) from a lipid layer 1.5 to 2 times excess of full coverage at the air/water interface. The technique for monolayer formation has been described earlier [8,9,11,12]. The layers are fluid, stable and are reproducible in their conformation and properties although purity of phospholipid is essential [8,9]. The coated electrode is subsequently held in the electrolyte phase. The $CO_2/NaHCO_3$ buffering system was used to preserve the pH at 8.1 and 6.2 [8]. The lipid layer is responsive to any impurity in the electrolyte. The lipid coating is readily renewed after each experiment, by raising the electrode, renewing the mercury drop and recoating the mercury surface. The electrode is connected to a potentiostat and is part of the conventional three electrode system. As a result, the capacitance of the monolayer can be measured using out of phase a.c. voltammetry and cyclic voltammetry. All potentials in this paper are reported versus Ag | AgCl 3.5 $mol \cdot dm^{-3}$ KCl.

The compounds used in this study were obtained from the following sources: DOPC from Lipid Products, U.K.; PAH, phenothiazines, biphenyl and DDT from the Aldrich Chemical Company; pyrethroids, allethrin and tetramethrin from the Greyhound Chemical Company; PCB were obtained from the Water Research Centre (J.W. Readman) except for 3,4-dichlorobiphenyl, 3,4-dibromobiphenyl, the tetrachlorobiphenyls and the hexachlorobiphenyls which were synthesized by one of us (J.T.B.).

The experimental technique for testing the effect of the organic compounds on the monolayer is to add a

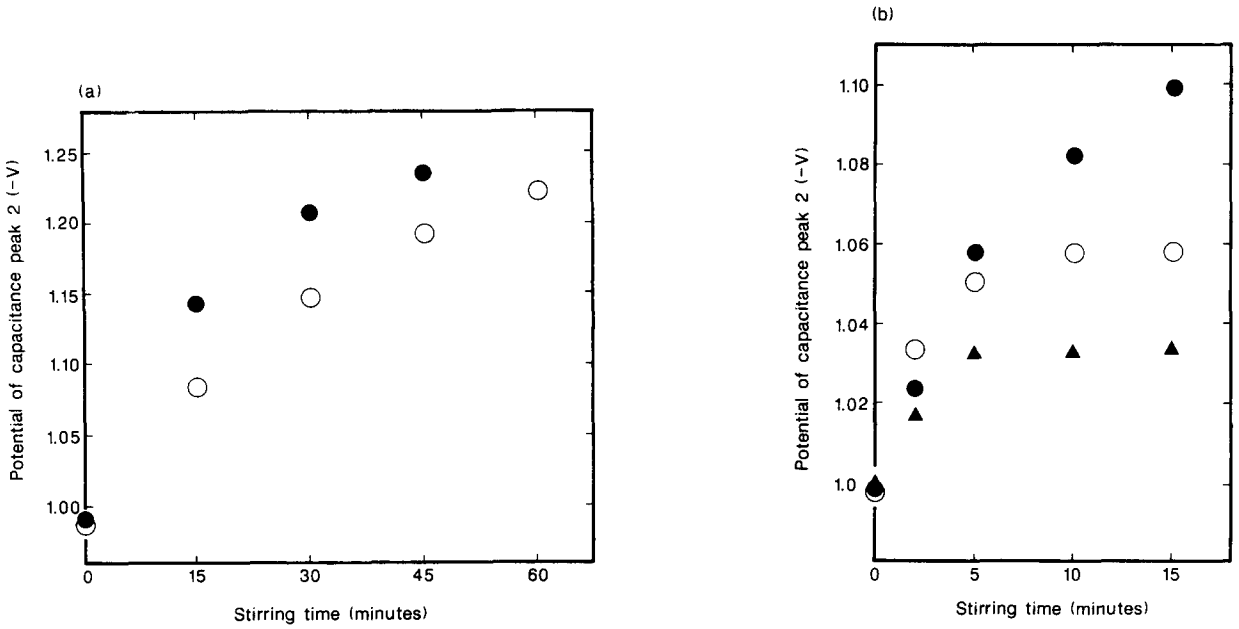
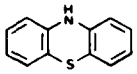
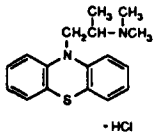
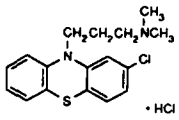
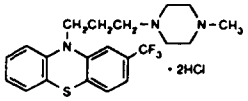
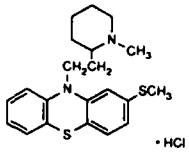


Fig. 1. Rate of interaction of hydrophobic species with the adsorbed DOPC monolayer. Response (expressed as capacitance peak 2 potential shift) versus stirring time. (a) PAH. ●, Benz[*a*]anthracene, $1.75 \cdot 10^{-8}$ mol·dm^{-3} in solution; ○, benzo[*a*]pyrene, $1.6 \cdot 10^{-8}$ mol·dm^{-3} in solution. Capacitance-potential curves measured by a.c. voltammetry. (b) PCB, $6 \cdot 10^{-8}$ mol·dm^{-3} in solution. ●, 2,4,5,3′-Tetrachlorobiphenyl; ○, 2,5,2′,5′-tetrachlorobiphenyl; ▲, 2,4,6,2′-tetrachlorobiphenyl. Capacitance-potential curves measured by cyclic voltammetry (40 V·s^{-1}).

TABLE I

Cationic phenothiazine drugs: physicochemical properties and activity on the adsorbed lipid layer

Compound	Structure	Critical micelle concentration (mmol·dm^{-3}) [ref. 24]	Concentration causing 50% depression of capacitance peak 1 of the adsorbed monolayer (CP_{50}) (μmol·dm^{-3})		pK 1	pK 2 [ref.17]
Phenothiazine			pH 8.1	pH 6.2		
Promethazine · HCl		43.8	3.50		9.4	
Chlorpromazine · HCl		18.6	0.45	1.05	9.3	
Trifluoperazine · 2HCl		9.8	0.22		8.1	3.6
Thioridazine · HCl		6.1	0.05	0.14	9.5	

small quantity of the compound dissolved in a suitable solvent to the electrolyte (0.55 mol · dm^{-3} KCl). PAH, PCB and pesticides were initially dissolved in acetone (HPLC grade) as working solutions. All interactions with the monolayer were carried out with the monolayer being held at −0.4 V. Following addition to the electrolyte and a period of stirring, the capacitance-potential curve is recorded. The rate of interaction is generally dependent on the water solubility of the organic substance [11]. Equilibration times of the smaller PAH with the monolayer are generally within 15 min [11] thus a 15 min stirring accumulation time was employed for these compounds. Fig. 1 displays examples of the rate of interaction of (a) some larger PAH and (b) PCB with the monolayer where the response is assessed as a potential shift of capacity peak 2 (see next section). It is seen that the 4-membered and the 5-membered PAH have longer equilibration times than 15 min. PCBs with up to four chlorine atoms have a shorter equilibrium time of adsorption. As a result a 5-min stirring time was used in this instance, however for 4- and 6-chloro-substituted PCBs, a 15-min equilibration time was also employed. The phenothiazine compounds were initially dissolved in methanol (HPLC grade) as working solutions and required only 1 min to equilibrate with the monolayer. The relatively fast equilibration times of the phenothiazines compared to those of the PAH and PCB relates to the higher water solubility of these compounds [11]. The arbitrary use of a fixed limit contact time means that for some compounds there is a kinetic contribution to the sensitivity of the monolayer to different compounds. Solutions are stirred prior to the recording of a capacitance curve so that during accumulation a stationary state is maintained at the electrode surface with a constant diffusion gradient across a diffusion layer. The reproducibility (standard deviation) of capacitance peak potential for a given peak shift has previously been given [12] and is within 4 mV. When % peak suppressions were recorded, the reproducibility expressed as total variation was within 3% peak suppression.

All PAH, PCB and pesticides were investigated below and up to the saturation solubility in water except for benzo[*a*]pyrene, 3-methylcholanthrene, chrysene, DDT and the hexachlorobiphenyls. With these compounds, concentrations in solution exceeded the saturation level. In previous studies of the comparative action of aromatic hydrocarbons on squid axon membranes [5], aqueous hydrocarbon concentrations have been expressed in terms of fractional saturation. This has not been possible in this study since there is a considerable discrepancy in the published saturation solubility values of the PAH and PCB. Table I illustrates the phenothiazines studied and their structures and properties. Phenothiazine p*K* values [17] (Table I) indicate that at pH 8.1; promethazine, chlorpromazine and thioridazine are more than 90% ionised. Trifluoperazine is 50% ionised. At pH 6.2, all cationic phenothiazines are more than 99% ionised. The acetone and methanol used as solvents were shown to have no effect on the monolayer at the concentrations added.

Experiments were also performed to assess the effect of known mole fractions of hydrophobic compound in the adsorbed monolayer on the capacitance properties of the monolayer. This was carried out by preparing mixed solutions of compound and lipid in pentane. These solutions were spread at the air/water interface and subsequently deposited on the HMDE. Since we assume the mole fractions of compound deposited on the electrode to be the same as the mole fraction of the compound in the spreading solution, we call this the apparent mole fraction of compound in the layer. From these experiments, the effect of the apparent mole fraction of the compound in the monolayer can be compared with the effect of the same compound when it penetrates from solution. Thus mole fractions of the hydrophobic compound in the monolayer in equilibrium with concentrations in solution can be calculated. These experiments were carried out with the PAH, a few representative PCB and DDT.

Mercury-adsorbed DOPC monolayer properties

Classically the interfacial properties at an electrode surface are recorded as measurements of interfacial tension and differential capacitance [18]. The differential capacitance is the capacitance of the interface and is equal to the double differential of the interfacial tension with respect to potential [18]. When a surfactant material is adsorbed at the interface, the interfacial tension will be lowered and any sharp change in the curve of interfacial tension with respect to potential will be manifest as a capacitance peak [18]. Naturally, sharp changes in surface tension are accompanied by either desorption of the surfactant or a restructuring of the adsorbed material. In the case of phospholipid films adsorbed on mercury electrodes, the layer is insoluble and any capacitance peak represents a restructuring of the lipid layer.

Fig. 2 shows the differential capacity-potential curve of a monolayer of DOPC on mercury. In the capacity minimum region the lipid tail segments are adsorbed on the mercury and the head group segments are concentrated in the interface between hydrophobic tails and water. Due to the highly co-operative nature of the self-assembly process, the compact monolayer closely resembles a half bilayer of the lipid membrane and has a coverage of $3.2 \cdot 10^{-10}$ mol · cm^{-2} [9]. Only in the interface between the mercury and the first few segments of the lipid tails is there any perturbation of the monolayer structure [13]. The polar head/water interface will most likely have similar properties to its biological counterpart. In electrochemical terms the system

behaves as an ideal parallel plate capacitor with low dielectric hydrocarbon (dielectric constant = 2) sandwiched between two capacitor plates, mercury and electrolyte solution respectively [8]. The capacitance is directly proportional to the dielectric constant of the dielectric and inversely proportional to the thickness of the monolayer in the classical way [8]. Clearly any change in the nature of the dielectric due to a structural reorganisation will give rise to changes in capacitance accompanied by a capacitance peak. Thus at negative potentials there are two reversible capacitance peaks which correspond to a structural reorganisation accompanied by a sharp increase in permeability to some water soluble ions. At more negative potentials, a capacitance peak at −1.25 V corresponds to a breakdown of the layer [8,9].

Characteristically the mercury-adsorbed monolayer is sensitive to organic compounds dissolved in the electrolyte [12] and the response is manifest as an effect on the reversible capacitance peaks. The shape and position of these peaks are selectively and quantitatively sensitive to groups of organic compounds [12]. The two capacitance peaks have each been shown to represent well defined phase transitions dependent on a subtle adsorption energy balance of the phospholipid [13,14]. Indeed, since these phase transitions are also highly dependent on the self-assembly properties of the lipids, any disturbance of these properties due to the effect of additives is reflected in an alteration of the capacitance peaks. Interaction of compounds with the system are recorded around the Potential of Zero Charge (PZC) in the capacity minimum potential region. The PZC is the potential of the interface with respect to the reference electrode system where the electrical double layer of the capacitor is effectively discharged and is normally about

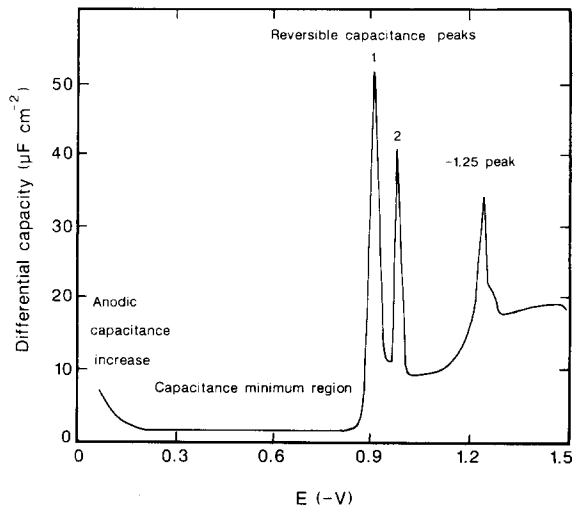


Fig. 2. Differential capacity-potential curve for an adsorbed monolayer of DOPC on mercury. Electrolyte, 0.55 mol·dm^{-3} KCl. Measurements by out of phase (90°) a.c. voltammetry; frequency, 75 Hz; amplitude, 0.01 V. Scan rate, 0.005 V·s^{-1}. (From Ref. 9.)

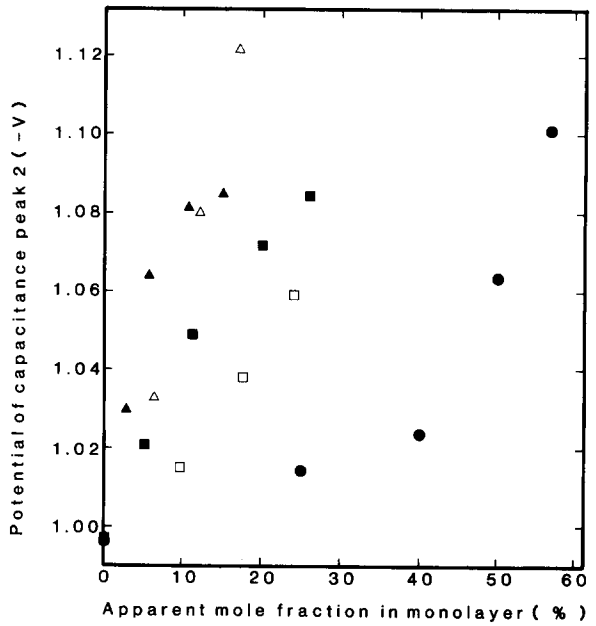


Fig. 3. Relationship between the capacity peak 2 potential and the apparent mole fraction % PAH in the phospholipid monolayer. ●, Pyrene; ■, benz[*a*]anthracene; □, chrysene; △, benzo[*a*]pyrene; ▲, 3-methylcholanthrene.

−0.45 V vs. Ag|AgCl on a mercury electrode in the absence of specifically adsorbing anions [18]. At the PZC, there is no potential drop across the monolayer.

Observations of changes in the nature of the phase transitions and consequently the capacitance peaks represent a very powerful way of monitoring the effect of additives in the system. In order for this approach to be validated, we must be sure that these capacitance peak changes correspond to the physical incorporation of material into the monolayer. In Fig. 3 we can see the response of the capacitance peak 2 potential to the apparent mole fraction of PAH in the monolayer. Note that for the higher membered less water soluble PAH the initial relationship between peak shift and mole fraction in the layer is linear. For some PAH this relationship curves at higher mole fractions of PAH and is probably related to the packing of the PAH in the layer. As regards pyrene, the initial slope in Fig. 3 is small. This is an artifact due to a solubility of pyrene in water decreasing the actual mole fraction of PAH in the air-water surface layer. We confirmed this by measuring a significant concentration of pyrene in the water in equilibrium with the air-water mixed surface layer using the lipid coated electrode [9]. In the case of pyrene in Fig. 3, only the final slope accurately represents the relationship between mole fraction in the layer and capacitance peak shift. These relationships will be discussed in detail in further studies since they do depend on the particular PAH in the monolayer. Notwithstanding, we can calculate from these results the mole fraction of compound in the layer which has penetrated the

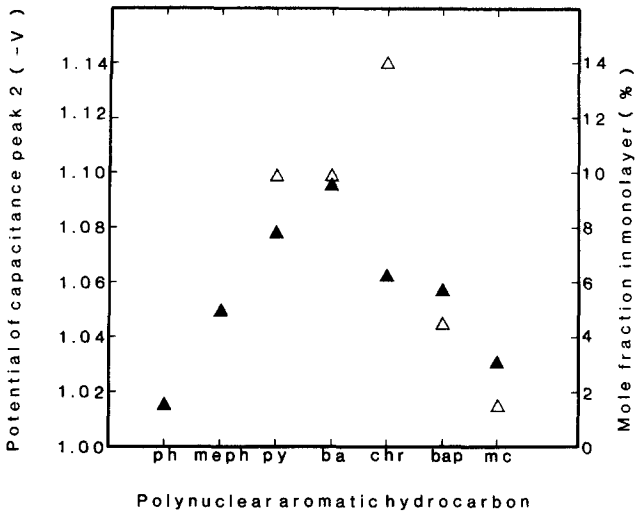


Fig. 4. Capacitance peak 2 potential of DOPC monolayer in response to (closed symbols), and % mole fraction of PAH in monolayer accumulated from [a] (open symbols) $1 \cdot 10^{-8}$ mol·dm^{-3} PAH in solution after 15 min stirring. ph, phenanthrene; meph, 1-methylphenanthrene; py, pyrene; ba, benz[*a*]anthracene; chr, chrysene; bap, benzo[*a*]pyrene; mc, 3-methylcholanthrene.

[a] estimated from the capacitance peak shifts from PAH penetration and the relationships in Fig. 3.

monolayer from a given concentration in solution under given conditions knowing the capacitance peak response in both instances (see Fig. 4).

Results

Polynuclear aromatic hydrocarbons

The interaction of the PAH with the monolayer has already been well studied [11,12]. One of the effects of their penetration is a negative shift in the potentials of the reversible capacitance peaks. The important features of the PAH molecule for monolayer response are: hydrophobicity, aromaticity and water solubility [11,12]. This is clearly shown in the order of capacitance peak shift following interaction with PAH [12] (Fig. 4). For the higher membered PAH, the order of monolayer affinity and the corresponding log K_{LW} of the PAH under the experimental conditions in Fig. 4 is: chrysene, 7.3 > benz[*a*]anthracene, 7.1 > pyrene, 7.1 > benzo[*a*]pyrene, 6.8 > 3-methylcholanthrene, 6.3. Although low water solubility plays a part in the decreased monolayer affinity of benzo[*a*]pyrene [11], it can be seen that after one hour's equilibration the monolayer is still more

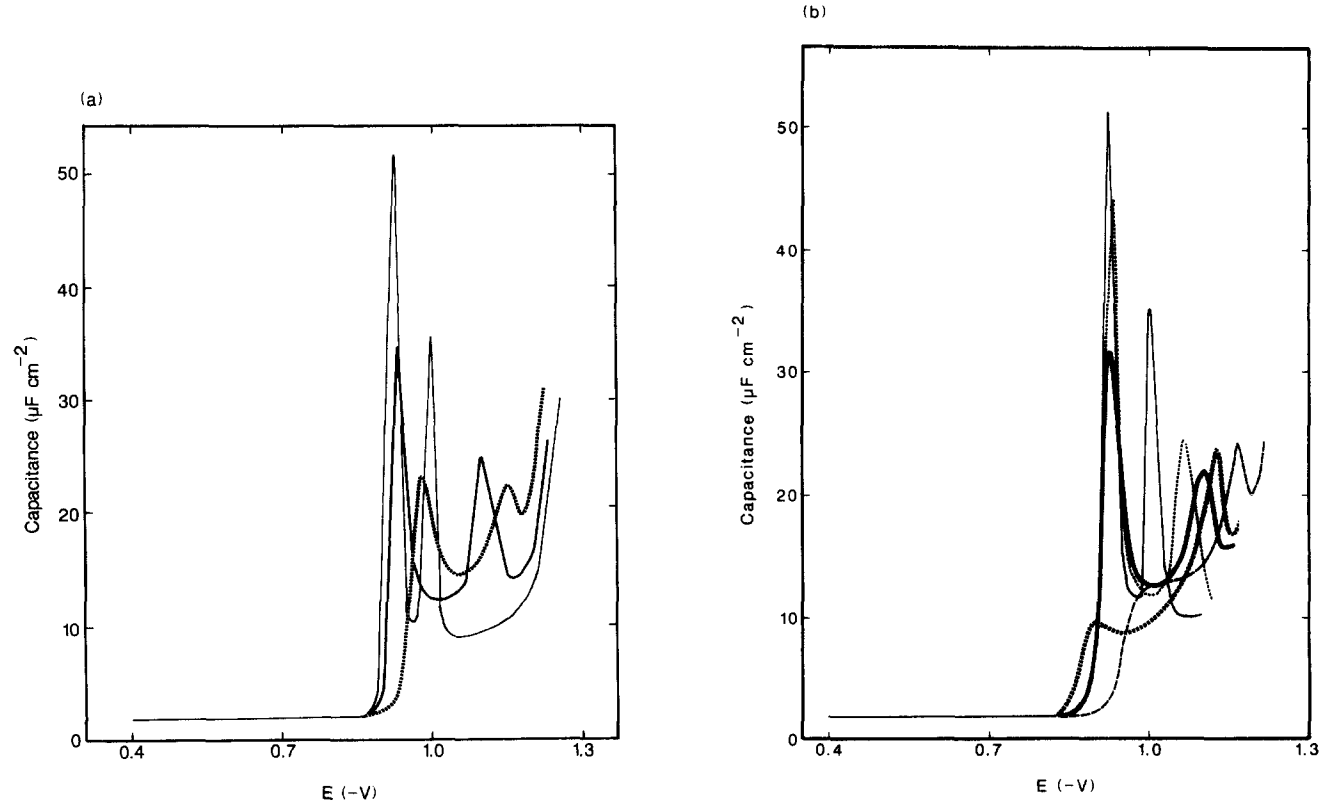


Fig. 5. (a) Effect of $2.7 \cdot 10^{-7}$ mol·dm^{-3} of 3,4-dichlorobiphenyl (- - - - - -) and 4,4′-dichlorobiphenyl (———), on the capacitance peaks of DOPC after 5 min stirring accumulation. Thin continuous line refers to the system prior to the addition of the hydrophobic compound. (b) Effect of $2.1 \cdot 10^{-7}$ mol·dm^{-3} of: 3,4,3′,4′-tetrachlorobiphenyl (- - - - -), 2,4,5,3′-tetrachlorobiphenyl (— — —), 2,5,2′,5′-tetrachlorobiphenyl (• • • • • •), 2,4,6,2′-tetrachlorobiphenyl (———), on the capacitance peaks of DOPC. 15 min stirring accumulation. The thin continuous line refers to the state of the system prior to addition of the hydrophobic compound.

sensitive to benz[*a*]anthracene than benzo[*a*]pyrene (Fig. 1a). It is proposed that the increased size of the 5-membered PAH hinders monolayer interaction. The dependence of the PAH affinity for the monolayer on the molecular properties of the PAH shows that processes of molecular adsorption and not aqueous diffusion are the limiting factors in the interaction of these compounds with the monolayer.

Polychlorinated biphenyls

The interest in PCB is twofold:

(1) These molecules vary in structure from planar to distorted to twisted depending on the substitution of the chlorine atoms. An *ortho*-chloro substituent will produce a distorted structure whereas two *ortho*-chloro atoms will bring the structure into a twisted conformation [19] with the aromatic rings at right angles. It was of great interest to see the effect of structure on monolayer permeation.

(2) PCB occur with varying degrees of chlorine substitution. Generally the effect of chlorine substitution is to increase the molecular polarisability of the molecule. This presented the opportunity of studying the effect of polarisability on monolayer interaction.

Figs. 5 and 6 display the results of the effect of PCBs on the mercury-adsorbed DOPC monolayer. Fig. 5 shows that the effect of PCB on the capacitance-potential curves of the adsorbed lipid monolayer is related to that of the PAH [11]. Biphenyl has a relatively low hydrophobicity (log $K_{OW} = 4.10$ [20] compare Table I from Ref. 11) and is planar, as a result its effect on the monolayer is small (see Fig. 6a). Substitution of chlorine atoms on to biphenyl greatly increases the monolayer response due to the increase in molecular polarisability. However the response is also dependent on the position of the chlorine atom and hence the planarity of the molecule. Indeed, the effect of *ortho*-chloro substitution is to depress sensitivity (Fig. 6a). Where the PCB molecule is planar with substitution only on one ring as in 3,4-dichlorobiphenyl, the response of the monolayer is identical to that of the PAH with a negative shift and depression of both capacitance peaks (Fig. 5a). Conversely when the chlorine substitution is on both rings (4,4′-dichlorobiphenyl) or is increased, one observes a negative shift and depression of capacitance peak 2 and only a depression of capacitance peak 1 (Figs. 5a and 5b). The sensitivity of response as measured by the extent of potential shift of capacitance peak 2 is correspondingly less (Figs. 6b and 6c). Also observed in Fig. 6b is the effect of bromine in 3,4-dibromobiphenyl in increasing sensitivity due to increased molecular polarisability. In Fig. 6c it can be seen that for the higher substituted PCB the sensitivity is not solely determined by the increased polarisability. This is offset by the molecular distortion (2,4,2′,4′-tetrachlorobiphenyl) and markedly by substitution of the 3,4-posi-

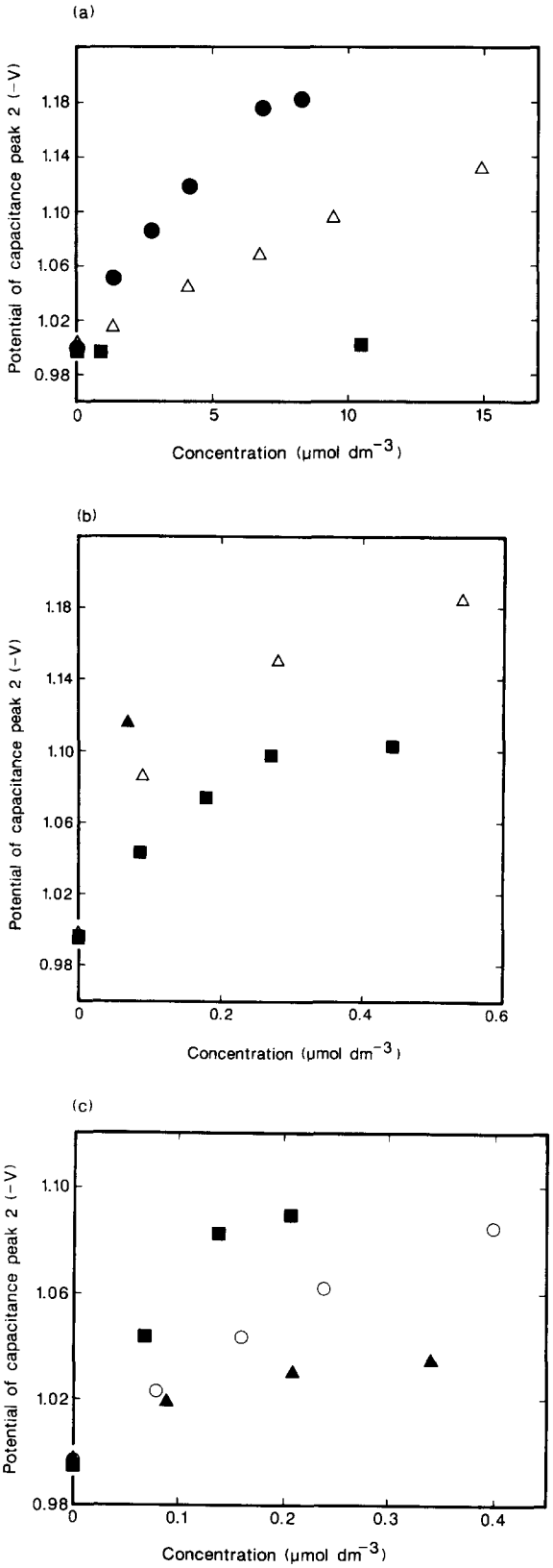


Fig. 6. Effect of concentration of PCB on the potential of capacitance peak 2 of DOPC. 5 min stirring accumulation. (a) ■, Biphenyl; △, 2-monochlorobiphenyl; ●, 4-monochlorobiphenyl. (b) ■, 4,4′-Dichlorobiphenyl; △, 3,4-dichlorobiphenyl; ▲, 3,4-dibromobiphenyl. (c) ○, 2,3,5-Trichlorobiphenyl; ■, 2,4,2′,4′-tetrachlorobiphenyl; ▲, 3,4,3′,4′-tetrachlorobiphenyl.

TABLE II

Effect of 4- and 6-chlorobiphenyls at $2.1 \cdot 10^{-7}$ mol·dm^{-3} concentration on the capacitance-potential curves of mercury adsorbed DOPC

PCB compound	Number of *ortho*-substituted chlorine atoms	Capacitance minimum value at −0.4 V [a] (μF·cm^{-2})	Potential of capacitance peak 2 [a] (−V)
3,4,3′,4′	0	1.80	1.065
2,4,5,3′	1	1.54	1.162
2,4,2′,5′	2	1.65	1.129
2,5,2′,5′	2	1.38	1.127
2,4,6,2′	3	1.76	1.108
2,3,4,6,2′,4′	3	1.80	1.084
2,3,5,6,2′,5′	3	1.80	1.100

[a] Recorded after a 15 min stirring accumulation.

tions on both rings with chlorine atoms (3,4,3′,4′-tetrachlorobiphenyl).

It is appropriate now to consider Table II where the shift in capacity peak 2 is tabulated for 0.21 μmol · dm^{-3} of the 4- and 6-substituted PCB in solution. We note two results: firstly, in the 4-substituted PCB the shift of capacity peak 2 is greatest for those compounds with the smallest number of *ortho*-substituted chlorine atoms (excepting 3,4,3′,4′-tetrachlorobiphenyl) and secondly the increasing chloro substitution of the PCB reduces sensitivity significantly. We also note from Table II that the capacitance minimum at −0.4 V decreases on penetration of the monolayer by PCB. The extent of this decrease partly correlates with the capacitance peak shifts. Although the PCB have a higher dielectric constant, 4–6 [21] than the hydrocarbon of the monolayer, this is more than counterbalanced by a capacitance decrease due to thickening of the layer. The concentrations of PCB in the monolayer which give rise to the capacitance peak shifts are the subject of further studies but generally a capacitance peak 2 shift of −0.06 V corresponds to 50% mole fraction PCB in the monolayer. Fig. 5b displays the influence of the 4-substituted PCB in the capacity peaks of DOPC. Both 3,4,3′,4′- and 2,4,6,2′-tetrachlorobiphenyl have the smallest effect. 2,5,2′,5′- and 2,4,5,3′-tetrachlorobiphenyl show a stronger response but in both instances although the peaks are equally suppressed, 2,4,5,3′-tetrachlorobiphenyl shows the greatest negative shift of both peaks. Peak suppressions are greater in response to the more bulky highly substituted PCB than to the PAH and planar PCB (c.f. Fig. 5a and Ref. 11, Fig. 1b). For 4-chlorobiphenyl, 3,4-dichlorobiphenyl and the PAH, there is an initial suppression of capacitance peak 1 for small negative shifts. The capacitance of this peak reaches a constant value for larger negative shifts of the peak. On the other hand, the non-planar PCBs exhibit a greater depression of the peak for less negative shifts and with the most bulky PCB, a positive shift of capacity peak 1 is observed (Fig. 5b).

These investigations show that PCB interaction with the monolayer is related to physicochemical factors important in penetration and molecular packing. Thus an increase of polarisability enhances the affinity of the molecule for the monolayer. On the other hand, if the PCB molecule penetrates the monolayer vertically, molecular deviations from planarity will physically hinder penetration and the consequent packing of the molecule in the monolayer. The evidence supports this and is commensurate with previous studies [22] which have shown that the interaction of PCBs with phospholipid bilayer membranes is strongly correlated with the chloro substitution at the *ortho* positions and consequently the planarity of the molecule. In connection with this, another impediment to interaction arises from substitution of chlorine atoms at positions where initial molecular penetration occurs. Assuming that the PCB molecule enters the lipid monolayer at the 3,4-positions, blocking of these sites on both aromatic rings with chlorine atoms will impede penetration. This explains the reduced sensitivity of the monolayer to 3,4,3′,4′-tetrachlorobiphenyl and 4,4′-dichlorobiphenyl.

Neurotoxic pesticides

p,p′-DDT and its congeners have a strong effect on the adsorbed monolayer. The influence of these compounds is to suppress the cathodic capacitance peaks (Fig. 7a). There is no increase in the capacity minimum value indicating that any penetration of the hydrocarbon region of the monolayer (*p,p′*-DDT has a higher dielectric constant than the lipid hydrocarbon) is offset by a thickening of the monolayer. The interaction with *p,p′*-DDT is irreversible, thus *p,p′*-DDT cannot be desorbed from the monolayer in the same way as the

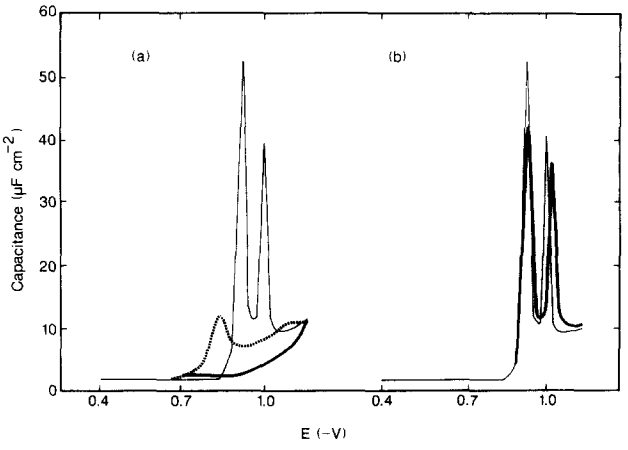


Fig. 7. (a) Effect of $1.1 \cdot 10^{-7}$ mol·dm^{-3} *p,p′*DDT and methoxychlor on the capacitance peaks of DOPC. 15 min stirring accumulation *p,p*-DDT (- - - - -). 60 min stirring accumulation, *p,p′*-DDT; 15 min stirring accumulation, methoxychlor (———). (b) Effect of $2.6 \cdot 10^{-7}$ mol·dm^{-3} endrin on the capacitance peaks of DOPC after 15 min stirring accumulation (———). The thin continuous line refers to the system's state prior to addition of the hydrophobic compound.

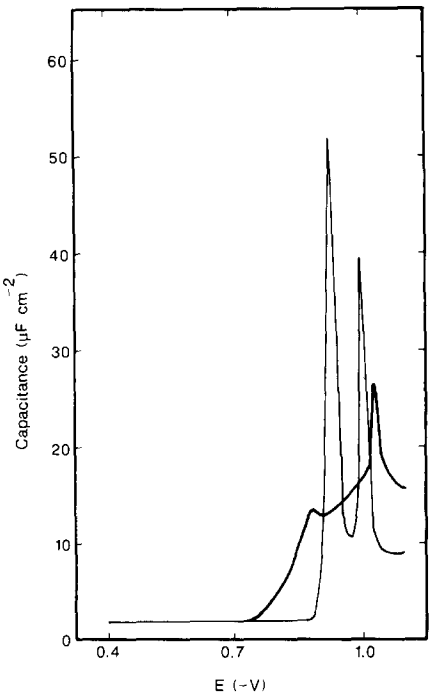


Fig. 8. Effect of $1.2 \cdot 10^{-7}$ mol·dm^{-3} thioridazine on the capacitance peaks of DOPC. 1 min stirring accumulation (———). The thin continuous line refers to the state of the system prior to the addition of the hydrophobic compound.

PAH [11]. The adsorbed lipid monolayer is sensitive to levels of p,p'-DDT down to 1 nmol · dm^{-3} and a mole fraction of 14% DDT in the monolayer is accumulated from 10^{-8} mol · dm^{-3} DDT in the electrolyte after 15 min stirring (log $K_{LW} = 7.3$). Thus the affinity of DDT for the monolayer is of the same order of magnitude as that of the four-membered PAH.

The DOPC coated electrode responds differently to the congeners of p,p'-DDT. Methoxychlor, which possesses two methoxy groups rendering it more hydrophilic and water soluble, has a faster action on the monolayer (Fig. 7a). The final effect of p,p'-DDT and methoxychlor is the same. The resemblance in equilibrium response to p,p'-DDT and methoxychlor shows that it is not the *para*-chloro groups which are critical in the adsorption of p,p'-DDT on the monolayer. Thus DDT has two non-coplanar aromatic rings together with a polarisable-CCl_3 group which must play a part in interaction.

The chlorinated cyclodiene insecticides; endrin, dieldrin and heptachlor, and lindane have comparatively little effect on the adsorbed monolayer system (Fig. 7b). A difference in action between DDT and the cyclodienes and lindane on black lipid membranes has been shown previously [23]. In addition, the pyrethroids, allethrin and tetramethrin have no observable effect on the monolayer at concentrations in the electrolyte similar to DDT. This is consistent with the other observations in this study since these compounds although hydrophobic possess no aromatic moiety.

Phenothiazines

Neutral phenothiazine has a small effect on the monolayer similar to that of acridine, a less hydrophobic and heterocyclic PAH and the capacitance peaks are shifted to negative potentials [12]. The change of the capacity peak characteristics in response to cationic phenothiazine drugs is different to neutral phenothia-

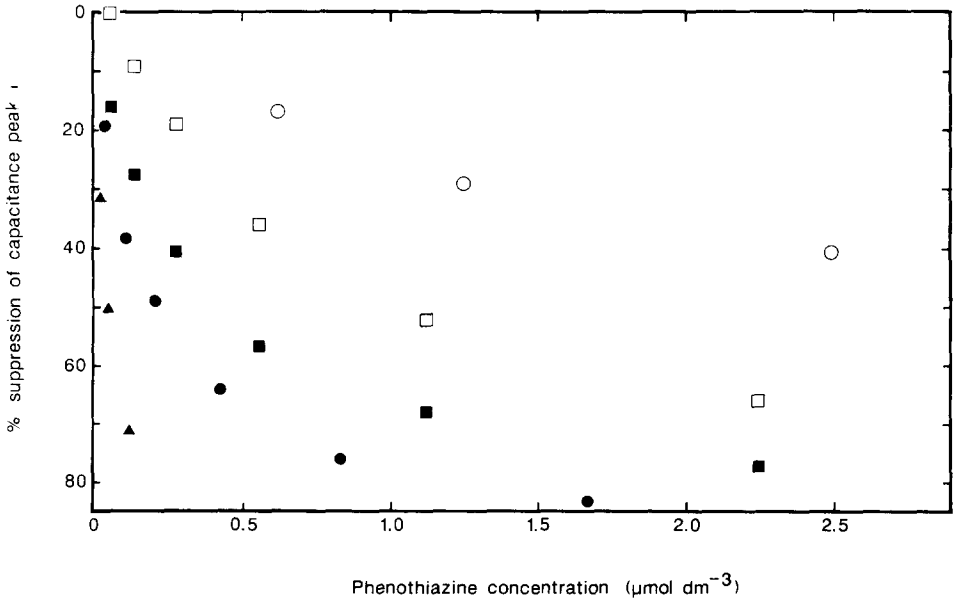


Fig. 9. Effect of cationic phenothiazine drugs on the height of capacitance peak 1. Percentage suppression versus the concentration of phenothiazine drug in solution. Height of peak recorded after 1 min stirring in solution. ○, Promethazine·HCl, pH 8.1; ■, chlorpromazine·HCl, pH 8.1; □, chlorpromazine·HCl, pH 6.2; ●, trifluoperazine·2HCl, pH 8.1; ▲, thioridazine·HCl, pH 8.1

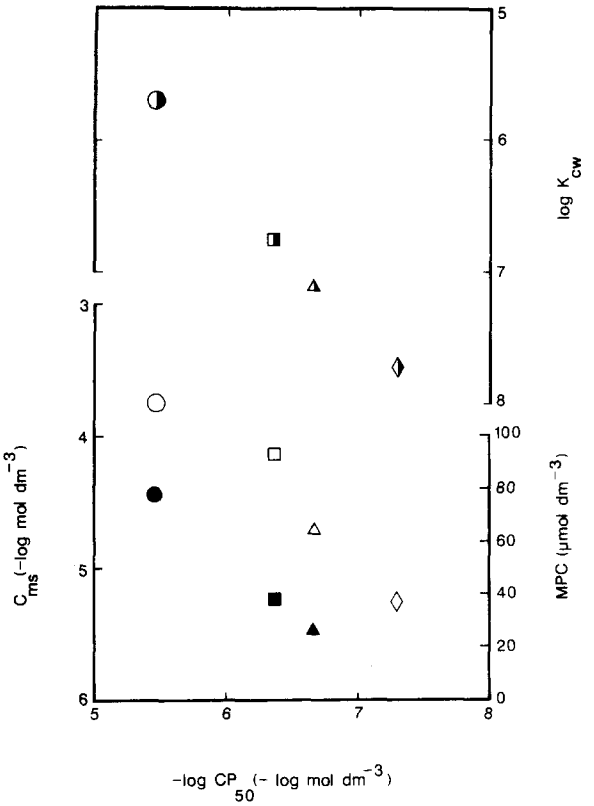


Fig. 10. Relationship between −log concentration of cationic phenothiazine inducing 50% suppression of capacitance peak 1 of DOPC adsorbed on mercury at pH 8.1 (−log CP_{50}) and the log(chloroform-water partition coefficient) for the unionised molecule (log K_{CW}) [21] (semi-closed symbols) or −log concentration of cationic phenothiazine (−log C_{ms}) giving maximum stabilisation of erythrocytes against lysis [23] (open symbols) or minimum protozoacidal concentration (MPC) of cationic phenothiazine to *Leishmania donovani* promastigotes [24] (closed symbols). ◑ ○ ●, Promethazine HCl; ◨ □ ■, chlorpromazine HCl; ▿ ▽ ▼, trifluoperazine 2HCl; ◈ ◇, thioridazine HCl.

zine and is manifest as a suppression and separation of the capacitance peaks (Fig. 8). There is no evidence to suggest competition by the phenothiazine for the metal surface since the capacitance minimum at −0.4 V is not altered and phenothiazine adsorption can be reversed by immersing the coated electrode (+adsorbed phenothiazine) in phenothiazine-free electrolyte.

The monolayer sensitivity to the phenothiazines follows the order: thioridazine > trifluoperazine > chlorpromazine > promethazine (Fig. 9). This is the same as the order of the surface activity of these drugs [24] (Table I) and correlates well with their lipophilicity expressed as the log chloroform-water partition coefficient, log K_{CW}, for the unionised molecule [17] (Fig. 10). The decreased sensitivity of the monolayer to these compounds in lower pH solutions (Fig. 9 and Table I) suggests that it is the uncharged molecule which is adsorbed (the monolayer itself is unaffected by this pH change [9]).

Discussion

It has been shown elsewhere that the two capacitance peaks correspond to phase transitions occurring in two steps in which the monolayer converts to a pored bilayer [13,14]. From fundamental interfacial electrochemical principles [25], we can say that a potential shift of the capacitance peaks corresponds to a change in the energetics of the phase transitions whereas a peak suppression and broadening indicates a lessening of co-operativity between the lipid molecules. The two phase transitions are mediated through a competition between the hydrophilic heads and hydrophobic tails of the lipid for the less hydrophobic mercury interface at negative potentials [13,14]. As a result any species which renders the heads more hydrophilic or the tails more hydrophobic will shift the capacitance peaks to a more negative potential. Clearly thus the negative shift of the capacitance peaks by PAH and planar low substituted PCB interaction is due to penetration of these compounds into the hydrocarbon region of the lipid layer increasing its extent of unsaturation and hydrophobicity. This is similar to the effect on the capacitance-potential curves of two phospholipids where the degree of unsaturation of one of the phospholipids has been increased [14]. It also explains the affinity of the monolayer for both aromatic and hydrophobic compounds.

It is clearly evident from this work that the planarity of aromatic compounds physically assists in penetration as shown with the PCB compounds. Thus as the PCB compound becomes more globular so the monolayer sensitivity to the compound is less and capacity peak 1 is increasingly interfered with manifested as a greater suppression (see Fig. 5b). The extreme of this effect is noted with the more highly substituted bulky PCBs and notably with DDT where a complete depression of both peaks is ultimately observed. These effects are quite complex but will relate to an increasing interference in a rather delicately balanced series of phase transitions, one effect being through the net increased coverage of the electrode which has a marked influence on the phase transition [14] and another being the removal of the co-operativity effect in the phase transition itself. Nonetheless increasing suppression and broadening of the capacitance peak will reflect increasing disruption of the self-assembly properties of the phospholipid monolayer and associated properties of permeability and fluidity. A similar effect has been observed when cholesterol is incorporated into the monolayer [9].

The effects of the charged cationic phenothiazines and DDT on the monolayer seems to relate partly to an increase in hydrophobicity of the head group which shifts the capacity peak 1 positively (Figs. 7 and 8). Hydrophobic cations at higher electrolyte concentrations have affected the capacity peak 1 similarly [9]. We note that in contrast to the PAH and PCB there is a

direct dependence of the phenothiazine interaction on their lipophilicity and the tricyclic part of the molecule is structurely identical in all phenothiazines examined. It is proposed therefore that the tricyclic moiety of the molecule penetrates the hydrocarbon region whereas the substituent chain remains in the polar phase. A similar orientation for anthraquinone-2-sulphonate in the monolayer has been proposed previously [10]. A comprehensive mechanistic understanding of these more complex interactions will be left for later studies. One clear finding from this work is the importance of molecular shape and size not only for phospholipid monolayer penetration but also for subsequent disruption effects of the monolayer properties of self-assembly. Also these effects can be readily monitored using the electrode-adsorbed monolayer system. Due to the similarity between the outer layers of the adsorbed monolayer and the phospholipid bilayer, we can expect that the interaction of these compounds with the lipid component of the biological membrane will operate at a similar level of selectivity. Indeed, properties of molecular size cut-off to hydrophobic organic compounds are inherent to lipid bilayers and are characteristic of the potency of anaesthetics [4].

Fig. 10 shows that in the experiment with the phenothiazines which are biologically active at the membrane level [24], there is a linear relationship observed between the adsorption of these drugs on to human erythrocytes [26] and their interaction with the adsorbed DOPC monolayer. Also observed is a correlation with the lethal activity of these drugs against *Leishmania* [27] which has been proposed as a membrane mediated event. The monolayer response to the neuroleptics is in accord with the biological membrane solubility of the drugs [28] and at the 10^{-8}–10^{-6} mol · dm^{-3} aqueous concentration, it is a sensitive assay for this property of the compound. At the same time the monolayer's affinity for these compounds is not due to lipophilicity alone since additional properties of the molecule promote interaction. Nonetheless in biological studies the lipophilic nature of these drugs has been found to be of major importance in aspects of their biological activity [17]. Whilst it is appreciated that the neuroleptic action of phenothiazines is more specific [29] than membrane adsorption alone, this result establishes a link between the selective sensitivity of the monolayer to organic compounds and their biological membrane activity.

Conclusions

Mercury-adsorbed phospholipid monolayers of DOPC are sensitive to the degree of hydrophobicity, aromaticity, planarity and size of hydrophobic organic compounds. The response of the monolayer to the compounds is manifested as a change in the form of the capacitance-potential curves especially with respect to two capacitance peaks which correspond to two well-defined phase transitions. Aromaticity, hydrophobicity and molecular shape determine the sensitivity of the response and the shape of the molecule also decides the type of response. Planar aromatic molecules cause a negative shift and broadening of the capacitance peaks however as the molecule becomes more globular the first capacity peak becomes more suppressed. The affinity of the PAH for the monolayer expressed as log K_{LW} respectively is in the order: chrysene, 7.3 > benz[*a*] anthracene, 7.1 > pyrene, 7.1 > benzo[*a*]pyrene, 6.8 > 3-methylcholanthrene, 6.3; thus there is a size cut-off for the higher membered PAH. The insecticide DDT has a high affinity for the monolayer (log $K_{LW} = 7.3$) and suppresses the capacitance peaks. The results indicate a molecular selectivity for these compounds by phospholipid monolayers which has implications for the effect of these compounds on biological membranes. It is shown that planar aromatic molecules exert their effect by penetrating into the hydrocarbon region of the monolayer. More bulky molecules have a disruptive effect on the phase transitions and consequently the mechanisms of self-assembly of the monolayers. A direct correlation is shown between the biological membrane activity of the phenothiazines and their effect on the monolayer at submicromolar levels. The results establish the mercury-adsorbed phospholipid monolayer system as a relevant model for assessing structure-biomembrane activity properties of organic compounds.

Acknowledgements

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References

1 Franks, N.P. and Lieb, W.R. (1982) Nature 300, 487–493.
2 Jain, M.K. and Wu, N.M. (1977) J. Membr. Biol. 34, 157–201.
3 Cohen, B.E. (1975) J. Membr. Biol. 20, 205–234.
4 Haydon, D.A., Hendry, B.M., Levinson, S.R. and Requena, J. (1977) Nature 268, 356–358.
5 Hendry, B.M., Elliott, J.R. and Haydon, D.A. (1988) Proc. R. Soc. Lond. B224, 389–397.
6 Seelig, J., Macdonald, P.M. and Scherer, P.G. (1987) Biochemistry 26, 7535–7541.
7 Davis, S.S., James, M.J. and Anderson, N.H. (1986) Faraday Discuss. Chem. Soc. 81, 313–328.
8 Nelson, A. and Benton, A. (1986) J. Electroanal. Chem. 202, 253–270.
9 Nelson, A. and Auffret, N. (1988) J. Electroanal. Chem. 244, 99–113.
10 Nelson, A. and Auffret, N. (1988) J. Electroanal. Chem. 248, 167–180.

11 Nelson, A. (1987) Anal. Chim. Acta 194, 139–149.
12 Nelson, A., Auffret, N. and Readman, J. (1988) Anal. Chim. Acta 207, 47–57.
13 Leermakers, F.A.M. and Nelson, A. (1990) J. Electroanal. Chem., in press.
14 Nelson, A. and Leermakers, F.A.M. (1990) J. Electroanal. Chem., in press.
15 Piskorska-Pliszczynska, J., Keys, B., Safe, S. and Newman, M.S. (1986) Toxicol. Lett. 34, 67–74.
16 Lund, A.E. and Naharashi, T. (1981) Neuroscience 6, 11, 2253–2258.
17 Holmberg, M. and Van der Kleijn, E. (1972) Eur. J. Pharmacol. 18, 139–147.
18 Bard, A.J. and Faulkner, L.R. (1980) Electrochemical Methods: Fundamentals and Applications, pp. 488–452, John Wiley and Sons, London and New York.
19 Cullen, J.M. and Kaiser, K.L.E. (1983) in QSAR in Environmental Toxicology (Kaiser, K.L.E., ed.), pp. 39–66, Reidel, Dordrecht.
20 Bruggeman, A., Van der Steen, J. and Hutzinger, O.J. (1982) J. Chromatogr. 238, 335–346.
21 Sawney, B.L. (1986) in PCBs and the Environment (Waid, J.S., ed.), Vol. 1, p. 53, C.R.C. Press Inc., Boca Raton, FL.
22 Reich, T., Depew, M.C., Marks, G.S., Singer, M.A. and Wan, J.K.S. (1981) J. Environ. Sci. Health A16, 65–71.
23 Hilton, B.D. and O'Brien, R.D. (1970) Science 168, 841–843.
24 Attwood, D. and Florence, A.T. (1983) Surfactant Systems: Their Chemistry, Pharmacy and Biology, pp. 124–228, Chapman and Hall, London and New York.
25 Frumkin, A.N. and Damaskin, B.B. (1964) in Modern Aspects of Electrochemistry, Vol. 3 (Bockris, J.O'M. and Conway, B.E., eds.), pp. 149–221, Butterworths, London.
26 Seeman, P. and Weinstein, J. (1966) Biochem. Pharmacol. 15, 1737–1752.
27 Pearson, R.D., Manian, A.A., Harcus, J.L., Hall, D. and Hewlett, E.L. (1982) Science 217, 369–371.
28 Seeman, P., Tittler, M., Tedesco, J. and Lee, T. (1977) in Biological Activity and Chemical Structure (Buisman, J.A.K., ed.), pp. 131–145, Elsevier, Amsterdam.
29 Seeman, P., Lee, T., Chau-Wong, M. and Wong, K. (1976) Nature 261, 717–718.