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INFLUENCE OF VARIOUS CATIONIC AMPHIPHILIC DRUGS ON THE PHASE-TRANSITION TEMPERATURE OF PHOSPHATIDYLCHOLINE LIPOSOMES

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Abstract—The influence of 16 cationic amphiphilic compounds from various pharmacological groups on the phase-transition temperature (T_t) of dipalmitoyl-phosphatidylcholine (DPPC) liposomes was investigated using the method of differential scanning calorimetry. All drugs, the hydrophobicity of which varied in a wide range, depressed T_t. Biphasic dose–effect curves were obtained when the reduction of T_t (ΔT_t) was plotted vs the molar ratio of drug/DPPC; beyond a plateau, T_t could again be reduced markedly by increasing the molar ratio. Concomitantly with the depression of T_t, the width of the transition peak changed in a characteristic way: it broadened during the (first) steep part of the dose–effect curves and became narrow like a control transition when the plateau of the dose–effect curves was reached. At still higher ratios the peak broadened again and eventually vanished, probably due to a detergent-like effect of the drug. Increasing hydrophobicity of the compounds shifted the dose–effect curves to lower molar ratios and enhanced the ΔT_t attained at the plateau phase. It is proposed that the different potencies of the drugs to depress T_t result from different binding equilibria between the compounds and DPPC membranes, the individual equilibrium being determined by hydrophobic attraction and electrostatic repulsion.

Many drugs share in common—independent of their specific therapeutic action—the physico-chemical property of being cationic amphiphilic in nature, i.e. the molecules contain a hydrophobic ring system and a short side chain with a protonized amino group. These catamphiphilic drugs bind to phospholipid membranes; the drug molecules intercalate between the lipid molecules: the cationic group is placed between the polar head groups of the phospholipids, while the hydrophobic portion is directed towards the hydrophobic interior of the membrane [1–6]. The incorporation of drug molecules affects the physico-chemical properties of the lipid bilayer such as the phase-transition temperature (T_t) from the gel to the liquid-crystalline state. Below T_t the fatty acid chains of the phospholipids are stiff and packed in a highly ordered array; above T_t the chains are disordered and possess more motional freedom (for review see [7, 8]). In several publications it has been described that certain drugs from different pharmacological groups, e.g. phenothiazines [4, 5, 9–11], tricyclic antidepressants [12, 13] and local anaesthetics [9–11, 14, 15], depress the phase-transition temperature of liposomes prepared from different phospholipids. In spite of these numerous reports, a comparison of the dose-dependent efficiency of the investigated drugs seemed hardly possible, since the aspects governing the studies and the experimental conditions varied widely. The aim of the present investigation was, therefore, to compare under identical conditions the effects of several cationic amphiphilic compounds from various pharmacological groups on the phase-transition temperature of membranes from phosphatidylcholine (lecithin), applying the technique of differential scanning calorimetry. All drugs investigated lowered T_t; for the majority of the compounds biphasic dose–effect curves exhibiting a plateau were obtained. A comparison of the dose–effect curves reveals that the drugs not only possess a different affinity to the phospholipid but also a different potency to depress T_t corresponding to the plateau. A preliminary account of this study has been given elsewhere [16].

MATERIALS AND METHODS

1,2 - Dipalmitoyl - *sn* - glycero - 3 - phosphocholine (DPPC, purity greater than 99%) was purchased from Sigma (München, F.R.G.) and used without further purificiation. The drugs were applied as hydrochlorides except for quinidine-sulfate and chloroquine-phosphate.

Preparation of liposomes. The liposomes were prepared in the presence of the drugs as described by Cater *et al.* [12]. At a temperature of 4°, DPPC (about 5 mg) and the drug, both dissolved in chloroform (Merck, Darmstadt, F.R.G.), were given in a glass vial. Only chloroquine had to be added dissolved in buffer; control experiments revealed that the effect of a drug was independent of whether it was added in chloroform before resuspension or dissolved in the resuspension medium. At 20°, chloroform was evaporated under a stream of nitrogen for 2.5 hr. The samples were further dried overnight under a vacuum for 12 hr. After adding 100 μl of a

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14 mM TES*–histidine buffer adjusted with HCl to a pH 6.0, the precipitate was resuspended by shaking in a water bath for 2 hr at 50°, i.e. a temperature well above the T_t of DPPC. The pH of 6 provided an almost complete protonation of all the drugs investigated. Every 30 min the samples were placed for about 10 sec on a bench vibrator to achieve careful mixing.

Determination of the transition temperature. The calorimetric analysis was performed by a Perkin–Elmer DSC 2C differential scanning calorimeter. The probe is heated together with a blank at a programmed constant heating rate. In a feed-back loop the temperature of each sample is continuously controlled and the energy flow into each sample is immediately adjusted to yield the indicated temperature. In a thermogram the difference between the heat flows into both samples is plotted vs the increasing temperatures. At the endothermic transition from the gel to the liquid-crystalline state the DPPC-containing probe takes up an extra amount of energy compared with the water-containing blank. This additional heat flow is reflected by a deviation of the curve from the base line. The temperature of this transition can be taken from the temperature axis of the thermogram.

Ten μl of the drug-containing liposome suspension was placed in a stainless-steel capsule (Perkin–Elmer) and measured in the DSC 2C against a blank containing 10 μl of distilled water. The samples were heated from 7° to 52° with a rate of 5°/min at a sensitivity range setting of 0.5 mcal/sec. As shown by repeated runs the thermograms were highly reproducible. In order to evaluate T_t a straight line was fitted to the upward deflection of the transition curve; the intersection of this line with the base-line of the thermogram was projected to the temperature axis. The temperature scale was calibrated using indium (Perkin–Elmer, Überlingen, F.R.G.) and cyclohexane (reference substance for gas chromatography, Merck) as standards; the calibration was checked before each experiment. The depression of the transition temperature (ΔT_t) induced by a drug was the difference of the T_t of the drug/DPPC mixture and the T_t of the control containing DPPC only. The quantitative relationship between the drugs and DPPC is given as molar ratio. The actual free concentration of a drug could not be calculated properly because unknown amounts of drugs were bound to DPPC membranes.

RESULTS

Calorimetric determinations of pure DPPC liposomes yielded transition curves as shown in Fig. 1. The main transition occurred at $T_t = 41°$, thus matching values reported in the literature [4, 5, 11, 12, 17, 18]. The small pretransitional endotherm had an onset temperature of 32°, which is also similar to the reported data [4, 12, 17, 18].

The effect of adding increasing amounts of a catamphiphilic drug is illustrated using chlorphentermine as an example (Fig. 1). The pretransition was abolished even at low molar ratios; this finding is in accordance with observations of other groups [4, 12–14]. In the present paper the interest will be focused on the main transition. The drugs influenced both the temperature (T_t) and the shape of the main transition.

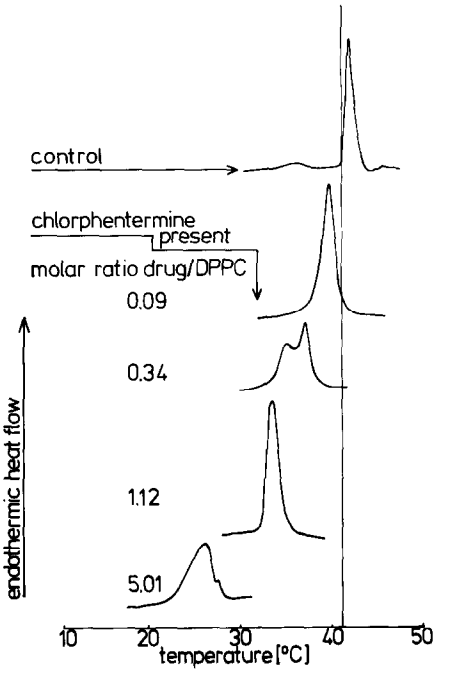

Fig. 1. Effect of the cationic amphiphilic chlorphentermine on the phase-transition endotherm of dipalmitoyl-phosphatidylcholine (DPPC) liposomes measured by differential scanning calorimetry. The samples were heated at a constant rate of 5°/min. Abscissa: temperature of the sample; ordinate: energy uptake by the sample. The amount of chlorphentermine added to DPPC is given by the molar ratio of drug/DPPC [mole/mole]. Chlorphentermine induced a downward shift of the temperature of the gel to the liquid-crystalline transition and a biphasic change of the shape of the peak.

T_t became increasingly depressed as the molar ratio of drug/DPPC was increased. In Fig. 2A the depression of T_t (ΔT_t) is plotted vs the molar ratio of chlorphentermine/DPPC. In the range of 0.6–1.6 mole/mole the dose–effect curve attained a plateau with ΔT_{plat} of about 10°; still further increasing the ratio, the dose–effect curve again rose steeply. In order to allow an estimate of the concentations in which the free, unbound chlorphentermine will probably have ranged, it should be mentioned that binding of [^{3}H]chlorphentermine to egg yolk phosphatidylcholine was characterized by a half-saturation concentration of 1.3×10^{-4} M [19].

The shape of the transition curve was affected in a biphasic manner (Fig. 1). With increasing molar ratios the signals became broader, developed two clearly distinguishable peaks and eventually narrowed to obtain a shape similar to a control transition. Comparable observations were reported by Cater *et al.* [12] and Frenzel *et al.* [4]. The double peak was interpreted to represent a two-component

* *N*-Tris (hydroxymethyl)-methyl-2-amino-ethanesulfonic acid.

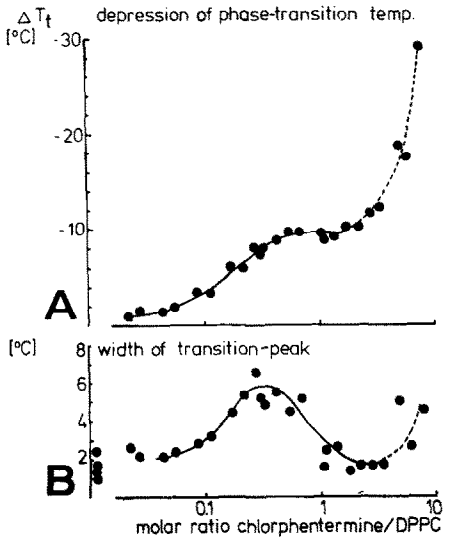

Fig. 2. Dose–effect curves presenting the effect of chlorphentermine on the onset temperature (T_t) of the phase transition (A) and on the width of the transition peak (B) as a function of the molar ratio (abscissa). The points represent individual determinations. (A) The depression of T_t equals the difference between T_t in the presence and T_t in the absence of the drug. (B) The peak width is given by the temperature range between the onset and the offset temperature of the transition endotherm. Note that the peak attains the width of a control transition (B) when the dose–effect curve describing the depression of T_t has reached a plateau (A). Further addition of drug induces a concomitant rise of both dose–effect curves, which is shown by dashed lines.

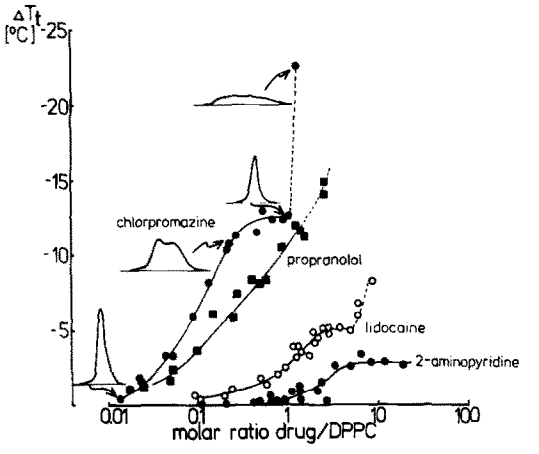

Fig. 3. Influence of chlorpromazine, propranolol, lidocaine and 2-aminopyridine on the transition temperature of DPPC liposomes. Abscissa: molar ratio of drug/DPPC [mole/mole]; ordinate: depression of the onset temperature of the phase transition. In the case of chlorpromazine some characteristic peak forms are presented to demonstrate the association between the plateau of the dose–effect curve and the narrowing of the peak. Beyond the plateau the curves are depicted as dashed lines; in the case of propranolol the dashed line starts when the liposome suspension became clear.

system with phosphatidylcholine molecules being surrounded by a higher and a lower density of drug molecules, respectively. Finally, with the incorporation of additional drug molecules all DPPC molecules were uniformly affected in a one-component system yielding a narrow peak. A further increment of the molar ratio again broadened and flattened the transition curve (Fig. 1). When the width of the peak, i.e. the temperature range between the onset and the offset of the transition, was plotted vs the molar ratio, the concentration-dependent variation of the shape of the transition became clearly apparent (Fig. 2B). Figure 2 also reveals a relationship between the effects of chlorphentermine on the temperature and on the shape of the transition. Parallel to the increasing depression of T_t the peak width broadened; when the dose–effect curve approached its plateau, the peak narrowed again and attained a shape at the end of the plateau similar to the control transition signal. At still higher dose ratios, the dose–effect curve and peak width rose again. The majority of drugs, which revealed a plateau in the dose–effect curves, showed a similar coincidence (see also Fig. 3); this was the more obvious the higher the potency of a compound to depress the T_{plat}.

Additionally, the appearance of the phospholipid suspension changed characteristically depending on the amount of chlorphentermine added. Under control conditions the DPPC suspension was milky. Addition of chlorphentermine in molar ratios up to that inducing the plateau slightly increased the viscosity of the suspensions; multilamellar liposomes of different size were visible by light microscopy. At a molar ratio of 5, i.e. beyond the plateau, the suspension had a jelly-like viscosity; after 6 hr of incubation at 50° the sample had become clear; light microscopically, liposomes were absent; the calorimetric signals flattened considerably in repeated runs. At a molar ratio of 10 the suspension had a consistency and transparency like water; by light microscopy liposomes were not detectable and phase transitions ceased to occur. The physical properties of the liposomal suspensions and the concomitant changes of the phase-transition signals were similarly influenced by most of the catamphiphilic drugs. Obviously, the phosphatidylcholine membranes were dissolved at high drug concentrations. A similar detergent-like effect of high concentrations of catamphiphilic drugs has been reported for tetracaine [20] and for a phenothiazine derivative [5]. It has been explained by a formation of mixed micelles composed of drug and phosphatidylcholine molecules [5, 20].

Since the dose–effect curve beyond the plateau can be associated with a detergent-like effect of drugs, the underlying mechanisms responsible for the second part were presumably completely different from the interaction between drug and phosphatidylcholine membrane causing the first part of the dose–effect curve up to the plateau. The dose–effect curves beyond the plateau are depicted as dashed lines (Figs. 2 and 3). Consequently, we will focus on the first part of the dose–effect curves. In Fig. 3 the dose–effect curves of four pharmacologically different compounds are shown. Additionally,

in the case of chlorpromazine some characteristic transition signals are depicted. The drugs have different potencies to reduce T_t: (a) the molar ratio necessary to depress T_t by 2° increased in the order chlorpromazine < propranolol < lidocaine < 2-aminopyridine; (b) ΔT_{plat} decreased in the order chlorpromazine > lidocaine > 2-aminopyridine. The dose–effect curve of propranolol did not reveal a plateau; since the suspension had become clear at a molar ratio of 1.2, the curve is shown as dashed line above this ratio. The total concentration of propranolol at this molar ratio was about 85 mM; it is remarkable that the critical micellar concentration of propranolol was reported to occur at about 100 mM [21].

With respect to local anaesthetics the molar ratio required for $\Delta T_t = 2°$ increased in the order dibucaine < tetracaine < lidocaine. As in the case of propranolol, a plateau could not be defined in the dose–effect curves of dibucaine and tetracaine. The reason might be that the detergent-like effect of these drugs occurred at too low a molar ratio, thus prohibiting the formation of a clearly discernible plateau.

For the sake of structure–activity relationship, the effects of four drugs with a chlorine substitution in the aromatic ring system were compared with the effects of their respective non-chlorinated compounds. In Fig. 4 the dose–effect curves are presented including the plateau. It is apparent that the introduction of a chlorine atom enhanced the potency of a catamphiphilic drug to depress ΔT_{plat}.

DISCUSSION

The phase-transition temperature of a phospholipid membrane does not depend only on the properties of the fatty acid chains but also on the nature of the polar head groups, e.g. the T_t of a phosphatidylethanolamine is higher than the T_t of a phosphatidylcholine with identical fatty acid chains

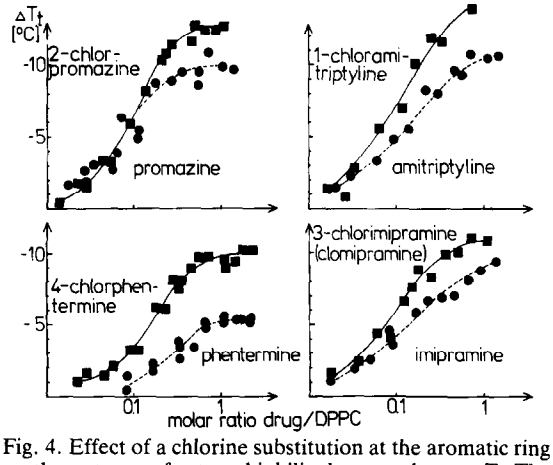

Fig. 4. Effect of a chlorine substitution at the aromatic ring on the potency of catamphiphilic drugs to depress T_t. The dose–effect curve of the chlorinated drug is compared with the dose–effect curve of the respective 'native' compound (dashed line). The second part of the dose–effect curves beyond the plateau—probably related to a detergent-like effect—has been omitted.

[17, 22]. In the case of DPPC, Chapman and coworkers found T_t to be influenced by the degree of hydration of the head groups [23] or by the interaction with the divalent cation O_2^{2+} [18]. Accordingly, the intercalation of cationic amphiphilic molecules between the polar head groups of a phospholipid membrane is able to depress its T_t.

The dose–effect curves for the influence of a number of catamphiphilic drugs on the T_t of DPPC membranes found in this study demonstrate that the drugs have different efficacies to depress T_t. The presented dose–effect curves cover a dose range of about 100-fold, suggesting different affinities to the phospholipid. Most of the dose–effect curves demonstrate a plateau. Since the second part of the dose–effect curve beyond the plateau is apparently related to a detergent-like effect, the different ΔT_{plat} reached at the end of the first part can be taken as a measure of different maximum efficacies of the drugs as long as the membranes are obviously intact. In an attempt to gain some insight into the mechanism determining this potency, the introduction of a chlorine atom into the aromatic ring was investigated, since it is well known that this substitution renders the compounds more hydrophobic [24]. Compared with the native drugs, the chlorine-substituted compounds had an enhanced potency. This finding is in accordance with results published by Seydel *et al.* [13]. In order to test whether the hydrophobicity of a catamphiphilic drug might be generally related to its potency, ΔT_{plat} as a measure of potency was plotted vs the octanol–water partition coefficient of the uncharged drug (Fig. 5). Only the drugs with a clearly discernible plateau and a known partition coefficient (compiled from [25]) were used. With a coefficient of $r = 0.92$, a rather good correlation was found between the hydrophobicity and the potency of the monovalent catamphiphilic drugs. In Fig. 5 also the effect of chloroquine is shown; unlike the other drugs chloroquine carries two positive charges at pH 6. Although it had a comparably high hydrophobicity, chloroquine was distinguished by a low potency. This finding suggests that electrostatic forces may determine the potency too.

In the following a hypothetical model is presented which attempts to explain the different potencies of the catamphiphilic drugs to depress T_t including data from the literature, particularly results we obtained in a study concerning the binding of radioactively labelled cationic amphiphilic drugs to liposomes of polar lipids [19]. Concepts describing anaesthetic drug action by a perturbation of cell membranes propose that local anaesthesia depends on the number of drug molecules introduced into the membrane (for review see [26]). Accordingly, we postulate that the extent of depression of T_t is only determined by the number of drug molecules intercalated into the phospholipid membrane, rather than by the molecular nature of the drug. The binding of the drug molecules to the zwitterionic, neutral phosphatidylcholine is assumed to be governed by two forces (Fig. 6): (a) the hydrophobic attraction; and (b) the electrostatic repulsion. A strong correlation has been found for the binding of radioactively labelled monovalent catamphiphilic drugs to phosphatidycholine liposomes and the hydrophobicity of the drugs [19];

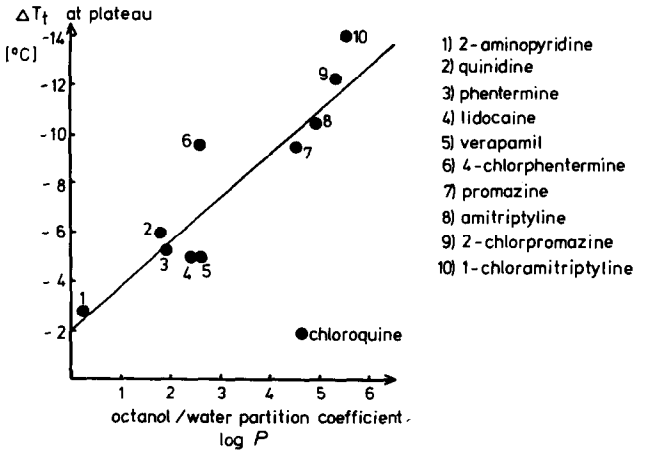

Fig. 5. Correlation between the potency of different cationic amphiphilic drugs expressed by ΔT_{plat} (ordinate) and the hydrophobicity expressed by the octanol–water partition coefficient (abscissa).

it was concluded that the hydrophobicity determined the extent of binding. The introduction of positively charged molecules into liposome membranes generates a positivation of the surface-potential of the membranes as shown by different authors [27–29]. As outlined by Lee [9, 10, 15], the positively charged surface repels catamphiphilic molecules, thus impeding further binding and yielding a saturation of the effect on T_t. Accordingly, the depression of T_t induced by the neutral local anaesthetic benzocaine was found to depend linearly on the concentration over the concentration range investigated [15]. When a certain amount of drug molecules is bound, the repulsive electrostatic force may neutralize the hydrophobic attraction, thus a further increase of the drug concentration in the medium cannot induce additional binding. A saturable binding of monovalent catamphiphilic drugs to phospholipid membranes had been directly demonstrated [19]. It is tempting to speculate that the plateau of the dose–effect curves found in the present study reflects the saturation of a binding process. The number of drug molecules bound and hence the maximum effect will depend on the relation between the opposing forces. Increasing the hydrophobic attraction, e.g. by chlorine substitution, will induce increased binding and an elevated plateau. This notion is supported by the observation that the maximum binding of chlorphentermine to phospholipid membranes is higher than that of phentermine [19]. Increasing the positive charge as in the case of chloroquine will enhance the electrostatic repulsion and diminish the effect on T_t. In fact, chloroquine was found to have a lower binding to liposomes from phosphatidylcholine than could be expected from its hydrophobicity [19]. The correlation shown in Fig. 5 demonstrates that the potency to depress the phase-transition temperature clearly depends on the hydrophobicity in case of monovalent cationic drugs.

In conclusion, the effect of cationic amphiphilic drugs on the temperature of the phase transition of DPPC liposomes is described by dose–effect curves, which revealed a different potency of the drugs to depress T_t. This different potency was independent of the main therapeutic action and could be explained by means of the physico-chemical properties, i.e. the charge and the hydrophobicity of the drug molecules.

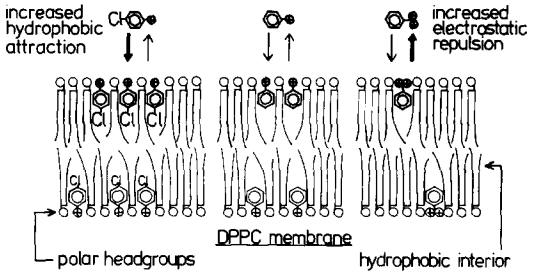

Fig. 6. Illustration of a hypothetical model to explain the different potencies of the catamphiphilic drugs to depress the phase-transition temperature of DPPC membranes. The depression of T_t is postulated to be dependent on the number of drug molecules incorporated into the membrane. The binding of a drug is thought to be governed by a hydrophobic attractive and an electrostatic repulsive force; for details see Discussion.

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REFERENCES

1. H. Hauser, S. A. Penkett and D. Chapman, *Biochem. biophys. Acta* **183**, 466 (1969).
2. J. Cerbon, *Biochim. biophys. Acta* **290**, 51 (1972).
3. P. L. Yeagle, W. C. Hutton and R. B. Martin, *Biochim. biophys. Acta* **465**, 173 (1977).
4. J. Frenzel, K. Arnold and P. Nuhn, *Biochim. biophys. Acta* **507**, 185 (1978).
5. M. Ahmed, J. Hadgraft, J. S. Burton and I. W. Kellaway, *Chem. Phys. Lipids* **27**, 251 (1980).
6. W. K. Surewicz and W. Leyko, *Biochim. biophys. Acta* **643**, 387 (1981).
7. D. Chapman, in *Form and Function of Phospholipids* (Eds. G. B. Ansell, J. N. Hawthorne and R. M. C. Dawson), p. 117, B.B.A. Library Vol. 3. Elsevier, Amsterdam (1973).

8. A. G. Lee, *Prog. Biophys. molec. Biol.* **29**, 5 (1975).
9. A. G. Lee, *Molec. Pharmac.* **13**, 474 (1977).
10. A. G. Lee, *Biochim. biophys. Acta* **514**, 95 (1978).
11. M. K. Jain, N. Y. Wu and L. V. Wray, *Nature, Lond.* **255**, 494 (1975).
12. B. R. Cater, D. Chapman, S. M. Hawes and J. Saville, *Biochim. biophys. Acta* **363**, 54 (1974).
13. U. Seydel, K. Brandenburg, B. Lindner and H. Moll, *Thermochim. Acta* **49**, 35 (1981).
14. D. Papahadjopoulos, K. Jacobson, G. Poste and G. Shepherd, *Biochim. biophys. Acta* **394**, 504 (1975).
15. A. G. Lee, *Biochim. biophys. Acta* **448**, 34 (1976).
16. B. Kursch and K. Mohr, *Naunyn-Schmiedeberg's Archs Pharmac.* **321**, R 35 (1982).
17. S. Mabrey and J. M. Sturtevant, *Proc. natn. Acad. Sci. U.S.A.* **73**, 3862 (1976).
18. D. Chapman, J. Urbina and K. M. Keough, *J. biol. Chem.* **249**, 2512 (1974).
19. H. Lüllmann and M. Wehling, *Biochem. Pharmac.* **28**, 3409 (1979).
20. M. Fernandez, *Biochim. biophys. Acta* **646**, 27 (1981).
21. D. Altwood and S. P. Agarwal, *J. Pharm. Pharmac.* **31**, 392 (1979).
22. A. Blume and H. Eibl, *Biochim. biophys. Acta* **640**, 609 (1981).
23. D. Chapman, R. M. Williams and B. D. Ladbrooke, *Chem. Phys. Lipids* **1**, 445 (1967).
24. A. Leo, C. Hansch and D. Elkins, *Chem. Rev.* **71**, 525 (1971).
25. H. Lüllmann, H. Plösch and A. Ziegler, *Biochem. Pharmac.* **29**, 2969 (1980).
26. P. Seeman, *Pharmac. Rev.* **24**, 583 (1972).
27. A. D. Bangham, K. R. Rees and V. Shotlander, *Nature, Lond.* **193**, 754 (1962).
28. M. A. Singer, *Biochem. Pharmac.* **26**, 51 (1977).
29. P. Schlieper, P. K. Medda and R. Kaufmann, *Biochim. biophys. Acta* **644**, 273 (1981).