CALORIMETRIC STUDY OF THE INTERACTION OF LOCAL ANESTHETICS [2-(ALKYLOXY)PHENYL]-2-(1-PIPERIDINYL)ETHYL CARBAMATES WITH MODEL DIPALMITOYLPHOSPHATIDYLCHOLINE MEMBRANES

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Local anesthetics of the homologous series of monohydrochlorides of [2-(alkoxy)phenyl]-2-(1-piperidinyl)ethyl esters of carbamic acid decrease the temperature of phase transitions $L_{\beta'} \rightarrow P_\beta$ (pretransition) and $P_\beta \rightarrow L_\alpha$ (main transition) of 1,2-dipalmitoyl-*sn*-phosphatidylcholine in aqueous phase at pH 6.2. The efficiency of the anesthetics in decreasing the pretransition temperature increases with the number of carbon atoms n of the alkoxy substituent up to $n = 6$. For $n > 6$ no pretransition could be detected. The efficiency in decreasing the temperature of the main transition increases up to $n = 8 - 9$ while it starts decreasing at $n = 10$. Computer simulation of the thermograms has shown that the decrease of efficiency at $n = 10$ is caused by the dependence of the partition coefficients of the anesthetics $K_{P,G}$ and $K_{P,LC}$ between the aqueous phase and the P_β and L_α phases, respectively, on the length of the alkoxy substituent, thus: $\log K_{P,LC} = A_{LC} + B_{LC}\, n$ and $\log K_{P,G} = A_G + B_G\, n$, where $B_{LC} < B_G$.

Lyotropic liquid-crystalline mesophases of lipids are widely used as models of the phospholipid membrane component. The effects of admixtures of amphiphilic molecules on these model systems should be studied not only to learn more about their physico-chemical properties but also to grasp the interactions of biologically active compounds with biological membranes[1].

Fully hydrated 1,2-diacylphosphatidylcholines form lyotropic liquid-crystalline phases which undergo the following phase transitions with increasing temperature: $L_{C'} \leftrightarrow L_{\beta'} \leftrightarrow P_\beta \leftrightarrow L_\alpha$, where the lamellar crystalline phase $L_{C'}$, the lamellar gel phase $L_{\beta'}$ and the rhombohedral gel phase P_β have their acyl chains mainly in the *trans*-configuration while the lamellar fluid phase L_α contains disordered acyl chains due to *trans–gauche* isomerization[2–7]. In the $L_{\beta'}$ and $L_{C'}$ phases the acyl chains are tilted at an angle with respect to the director (perpendicular with respect to the lamella) while in

the P_β phase they are parallel with the director. The surface of the lamella in the P_β phase is rippled[8–12]. The phase transition $L_{C'} \rightarrow L_{\beta'}$ is called subtransition, phase transition $L_{\beta'} \rightarrow P_\beta$ is the pretransition and phase transition $P_\beta \rightarrow L_\alpha$ is called the main transition.

In our earlier work we used spin probes and EPR spectroscopy[13] as well as polarization microscopy[14] to establish that tertiary amines exhibiting local anesthetic activities decrease the critical temperature of the main phase transition in 1,2-dipalmitoylphosphatidylcholine (DPPC) model membranes, this decrease being related to their anesthetic efficiency. In the present paper we describe the effect of local anesthetics of the homologous series of [2-(alkoxy)phenyl]-2-(1-piperidinyl)ethyl carbamates[15,16] (abbreviated C_nA where *n* is the number of carbon atoms in the substituent) on the pretransition and the main transition of DPPC. A part of the experimental results was published as a short communication[17].

EXPERIMENTAL

Reagents

1,2-Dipalmitoyl-*sn*-phosphatidylcholine was from Fluka (Buchs, Switzerland). Monohydrochlorides of [2-(alkoxy)phenyl]-2-(1-piperidinyl)ethyl carbamates were prepared as described before[15]. Other chemicals were of analytical purity from Lachema (Brno, The Czech Republic). The organic solvents and water were redistilled before use.

Sample Preparation

DPPC was mixed in an organic solvent with the corresponding amount of C_nA. The solvent was removed in a stream of nitrogen with subsequent evacuation in a two-stage oil vacuum pump for 5 h. The dry sample was hydrated with a buffer containing 103 mmol/dm^3 Na_2HPO_4 and 48.5 mmol/dm^3 citric acid in water and the pH of the sample was adjusted to 6.2. The resulting concentration of DPPC in the sample was 0.648 mmol/dm^3. Before measurement the samples were incubated for 1 h at 50 °C and were shaken several times during incubation and then equilibrated for at least 2 h at room temperature.

Equipment and Its Use

For the microcalorimetric measurements we used a differential adiabatic scanning microcalorimeter DASM-4 (Russian Academy of Sciences, Pushchino, Russia). The volume of the experimental and reference cells was 0.4789 and 0.4779 cm^3, respectively. To ensure complete filling of the cells and to prevent the presence of air bubbles in them, a constant external pressure of ≈177 kPa was applied. During the measurement we worked over the range of 20 – 50 °C, the rate of heating being 1 °C/min. The sensitivity of the calorimeter was 0.5 μW at the above rate of heating, the noise level was lower than the thickness of the recorder trace. The calorimeter was connected to an X–Y recorder and simultaneously, via an A/D converter, to a computer. At a constant rate of heating the temperature was read at 0.1 °C steps. Before the meaurement itself the baseline was recorded: Both the experimental and the control cell were filled at room temperature with the buffer used for preparing the samples. Both cells were cooled in a thermostat to about 10 °C and left to equilibrate for 5 min. Then the

heating was switched on. At about 18 °C the working regime was stabilized and the baseline was recorded. Together with the baseline a calibration was carried out by applying a precisely defined input of 50 μW for 5 min into the control cell. To record the thermogram of the studied sample we filled the experimental cell with the sample, leaving the buffer in the reference cell. Then we proceeded as when recording the baseline. The output of the experimental setup is the value of relative isobaric heat capacity c_P in dependence on the temperature with respect to the reference sample.

Evaluation of the Thermograms

The main transition temperature t_m (T_m) and pretransition temperature t_p (T_p) were defined as the temperature at which the dependence $c_P = f(t)$ reaches its maximum $c_{P,max.}$ While in the experimental results the temperature t is shown in °C, thermodynamic calculations require the use of absolute temperature T given in K. The calorimetric enthalpy ΔH_{cal} of the phase transition was determined by numerical integration on a computer as

$$\Delta H_{cal} = \int_{T_1}^{T_2} c_P \, dT \ , \tag{1}$$

where c_P is the relative heat capacity at constant pressure and T_1 and T_2 are the limits of the studied temperature interval. We integrated using steps of 0.1 K and less. Derivation was done numerically using a computer. Microcalorimetric literature usually defines the temperature-induced change of the material studied as the change from the original state A to the final state B, in analogy with the reaction

$$\begin{matrix} \mathrm{A} & \rightarrow & \mathrm{B} \\ (1-\alpha) & & \alpha \end{matrix} \ , \tag{2}$$

where α is the degree of conversion of state A to state B. van't Hoff's equation holds here:

$$[\partial(\ln K)/\partial T]_P = \Delta H_{VH}/\boldsymbol{R}T^2 \ , \tag{3}$$

where ΔH_{VH} is van't Hoff's enthalpy of the observed change, K is the equilibrium constant and $\boldsymbol{R}$ is the gas constant. On expressing $\partial(\ln K)/\partial T$ using the degree of conversion α we obtain

$$\Delta H_{VH} = [\boldsymbol{R}T^2/\alpha(1-\alpha)](\partial\alpha/\partial T) \ . \tag{4}$$

If the observed peak is symmetrical then at $T = T_m$ the same amounts of A and B coexist. Then

$$\Delta H_{VH} = 4\boldsymbol{R}T_m^2(\partial\alpha/\partial T)_{P,T=T_m} \ . \tag{5}$$

The degree of conversion can be expressed as the ratio of excess heat $Q(T)$ absorbed at T to the total excess heat Q_t absorbed during the whole process which, in the case of an isobaric arrangement, is equal to enthalpy ΔH_{cal}:

$$(\partial\alpha/\partial T)_P = (1/\Delta H_{cal})(\partial Q(T)/\partial T)_P = c_P/\Delta H_{cal} \quad . \tag{6}$$

Substitution into Eq. (*5*) yields

$$\Delta H_{VH} = 4\boldsymbol{R}T_m^2 c_{P,max}/\Delta H_{cal} \quad . \tag{7}$$

The van't Hoff enthalpy was calculated from this expression.

RESULTS AND DISCUSSION

Experimental Results

The lower part of Fig. 1 shows a thermogram for the system DPPC–aqueous phase at pH 6.2. The thermogram clearly displays two peaks. The one at the lower temperature is characterized by its greater width and lower intensity and corresponds to the transition $L_{\beta'} \rightarrow P_\beta$. The higher-temperature peak is markedly narrower and more intense and represents the main phase transition $P_\beta \rightarrow L_{\alpha.}$ Parameters of these transitions were evaluated from five measurements, the results being shown in Table I. Symbols t_m^0 and t_p^0 are temperatures at which the dependence of the heat capacity on temperature attains its maximum during the main transition and during the pretransition, respectively. The data of Table I are in fine agreement with those in the literature[7]. Figure 1 also shows thermograms of the DPPC–H_2O system in dependence on C_7A concentration at pH 6.2. There is a clear shift of the peak of c_P of the main transition to lower temperatures and its broadening with the increasing C_7A concentration. The pretransition is also shifted

TABLE I
Thermodynamic parameters of the main phase transition and the pretransition of the system DPPC–aqueous phase at pH 6.2

Transition	Parameter	Value
$P_\beta \rightarrow L_\alpha$	t_m^0, °C (K)	41.78 ± 0.06 (314.93)
	ΔH_{cal}^0, kJ/mol	29.76 ± 0.45
	$C_{p,max}^0$, kJ/mol K	34.4 ± 0.6
	ΔH_{VH}^0, kJ/mol	3 813 ± 20
$L_{\beta'} \rightarrow P_\beta$	t_p^0, °C (K)	35.93 ± 0.09 (309.8)

to lower temperatures and it becomes broader up to c_{LA} = 0.068 mmol/dm^3. At higher values of c_{LA} no pretransition could be detected.

Figure 2 shows the effect of the concentration of C_7A on the main transition temperature t_m and the pretransition temperature t_p. These dependences can be considered to be linear within the experimental error. Figure 3 shows the dependence of the maximum value of the molar heat capacity $C_{P,max}$ on the concentration of C_7A. This maximum is seen to decrease with increasing concentration of C_7A in a nonlinear way. The area under the $C_P = f(t)$ curve indicates the molar calorimetric enthalpy ΔH_{cal} (Eq. (*1*)). The concentration dependence of ΔH_{cal} for C_7A is shown in Fig. 4. The ΔH_{cal} value changes very little with concentration. The effect of the length of the alkoxy substituent of C_nA homologues on the form of the thermograms of the DPPC–H_2O system at c_{LA} equal to 0.136 mmol/dm^3 is shown in Fig. 5. There is simply a decrease of the temperature of the main phase transition and the broadening of the peak. To compare quantitatively the effect of the individual derivatives of the homologous series on the main transition temperature as was measured at different concentrations c_{LA}, we introduced the $\Delta t_m/c_{LA}$ parameter which normalizes the change of the temperature of the main phase transition to a unit concentration of the corresponding homologue C_nA in the sample. The dependence of $\Delta t_m/c_{LA}$ on the chain length is shown in Fig. 6. The data of the figure were obtained at c_{LA} = 0.136 mmol/dm^3 for $n \leq 5$ and at c_{LA} = 0.068 mmol/dm^3 for n = 6 – 10. It follows from the data of Fig. 6 that at an equal concentration of the admixture in the sample the efficiency of decreasing the main transition temperature is low for n = 1, 3, 5 while it increases sharply for n = 6, 7, 8 and reaches its maximum at n = 9. For n = 10 one may observe a decrease of this efficiency. The same procedure was used for the evaluation of the data for the pretransition. The dependence of $\Delta t_p/c_{LA}$ on the chain

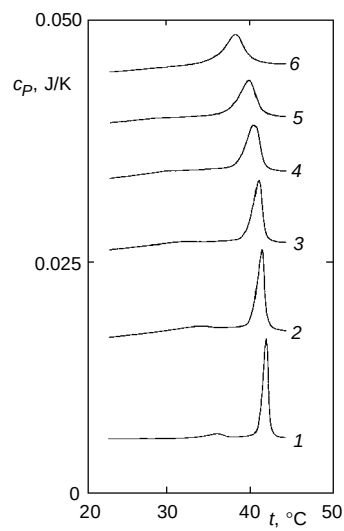

FIG. 1
Dependence of the heat capacity c_P of the measured sample (in J/K) on temperature in a system of DPPC–aqueous phase for different concentrations of C_7A (c_{LA}) at pH 6.2. Concentration c_{LA} is given in mmol/dm^3: *1* 0, *2* 0.017, *3* 0.035, *4* 0.068, *5* 0.103, *6* 0.136

length n shown in Table II indicates that the efficiency of decreasing t_p increases with n. For values of n higher than those in Table II no pretransition could be found.

The decrease of the critical temperature t_p of the pretransition in the presence of C_nA suggests an interaction of C_nA with lipids in the gel state. One can assume that the C_nA molecules are present in the P_β phase of the lipid, or even in the $L_{\beta'}$ phase where their number in P_β should be greater than in $L_{\beta'}$. The mechanism leading to the increased binding of C_nA to the P_β phase is not understood since both $L_{\beta'}$ and P_β are gel-type mesophases where the lipid chains are closely packed, predominantly in the all-*trans*-configuration. The only difference between them is in the fact that in the $L_{\beta'}$ phase the aliphatic chains are tilted to the membrane director while they are parallel with it in the P_β phase. One of the possible explanations is the different lateral packing of the lipid

TABLE II
Dependence of the $\Delta t_p/c_{LA}$ parameter on the length of the substituent n of C_nA homologues

n	$\Delta t_p/c_{LA}$, °C dm^3/mmol
1	4 ± 1
3	7 ± 1
5	12 ± 1
6	31 ± 3

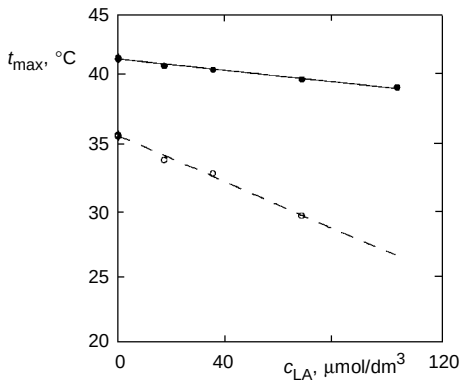

FIG. 2
Dependence of the temperature of the main phase transition t_m (———) and of the pretransition t_p (– – – –) in a system of DPPC–aqueous phase on concentration of C_7A (c_{LA}) at pH 6.2

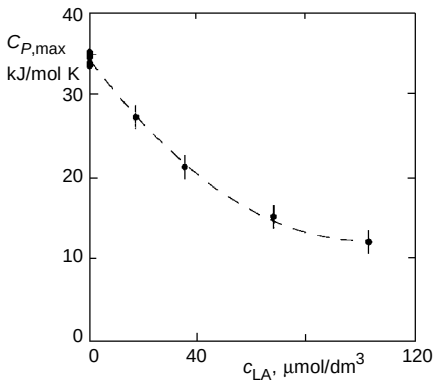

FIG. 3
Dependence of the maximum value of heat capacity $C_{P,max}$ on concentration of C_7A (c_{LA}) in a system of DPPC–aqueous phase at pH 6.2

molecules in these phases. The lipid bilayer is probably formed by domains of laterally closely packed lipids and by defects between such domains. The existence of domains and defects in the lipid bilayer was experimentally proved by the electrophoretic mobility of 1,2-diacylphosphatidylcholine liposomes[18] and by small angle neutron scattering on oriented 1,2-diacylphosphatidylcholine bilayers[19]. Moreover, a phase transition in a lipid bilayer is accompanied by the formation of domains of a new phase within the original matrix. The domain formation is a dynamic process and the domain size and its location near the phase transition temperature will fluctuate. This is the cause of the lateral heterogeneity of the bilayer[20,21]. In the gel phase of the lipid this domain structure is frozen because of the slow lateral diffusion of the lipid molecules. Under our experimental conditions the $L_{\beta'}$ phase reaches an equilibrium state after a relatively long time (2 h at least) while in the P_β phase the sample stays for only some 10 min. The P_β phase is thus probably more distant from the equilibrium state as compared with the $L_{\beta'}$ phase and hence will contain more lateral defects than the $L_{\beta'}$ one. Since these defects between the ordered domains are the sites of accumulation of admixtures[20,21] the present model of lateral heterogeneity might explain the increase in the number of bound molecules of C_nA in the P_β phase in comparison with the $L_{\beta'}$ phase and hence

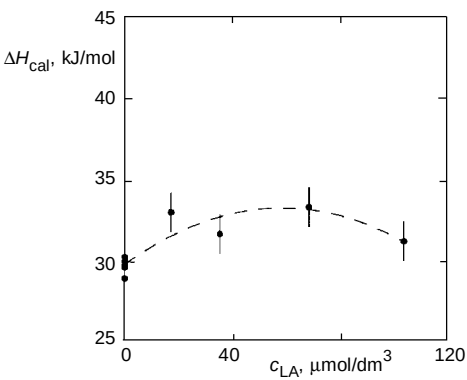

FIG. 4

Dependence of calorimetric enthalpy ΔH_{cal} on concentration of C_7A (c_{LA}) in a system of DPPC–aqueous phase at pH 6.2

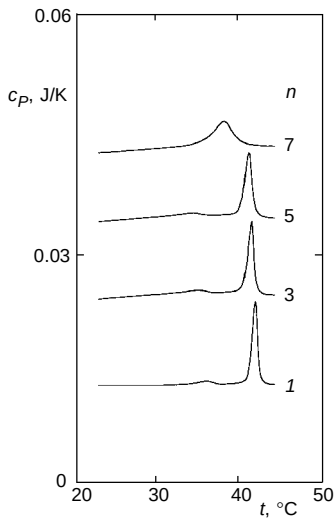

FIG. 5

Dependence of the heat capacity c_P of the measured sample (in J/K) on temperature for different homologues of C_nA in a system of DPPC–aqueous phase; *n* stands for the number of C atoms in the alkoxy chain. The bottom thermogram corresponds to pure DPPC. pH 6.2, c_{LA} = 0.136 mmol/dm^3

also a decrease in the value of t_p. Ueda et al.[22] recently found a decrease of t_p in a system of DPPC–aqueous phase in the presence of the tertiary amine local anesthetic lidocaine. They assume that the cause here is the different interaction of this admixture with the lipid–water interface in the $L_{\beta'}$ and P_β phases. Since these phases bind when fully hydrated different amounts of water molecules per molecule of DPPC the interfaces must be different. The authors suggest that the interaction of the admixtures with them will also show some differences. This might be another explanation for the observed effect. However, it is not clear how this model can take into account the increased efficiency of the C_nA homologues to decrease the t_p in dependence on the length of the substituent n at a constant concentration of C_nA in the sample. This dependence rather indicates a certain role of partition equilibria of C_nA between the lipid phase and the aqueous phase.

Some papers[23–28] described not only a shift and broadening but also a splitting of the C_P peak of the main phase transition of the lipid bilayers in the presence of amphiphilic admixtures but only at concentrations higher than those used here. Dorfler et al.[28] analyzed in detail the thermograms of the DPPC–C_7A–H_2O system up to a molar ratio of DPPC : C_7A = 1 : 1. They showed that the C_P peak of the main phase transition is split because of the existence of separated clusters with different molar ratios of DPPC to C_7A. On the basis of our results and the paper by Dorfler et al.[28] we assume that the C_nA molecules interact with lipid bilayers not only in the liquid-crystalline but also in the gel phase and in the region of concentrations examined in the present paper form a solid solution with the lipid. Above a certain limiting concentration, the value of which can depend on n, gel-type clusters form with molar ratios of DPPC : C_nA different from the surrounding solid solution. The temperature of the phase transition gel–liquid crystal is lower for these clusters than for the surrounding solid solution.

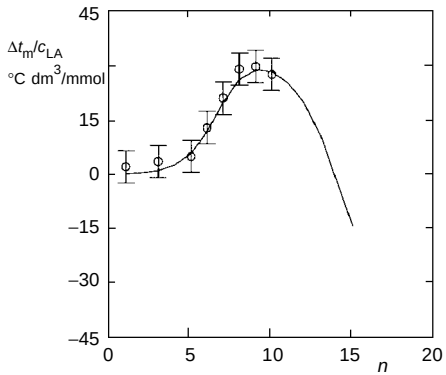

FIG. 6
Dependence of $\Delta t_m/c_{LA}$ on the length of the substituent n of C_nA homologues in a system of DPPC–aqueous phase. Circles: experimental points; curve: result of computer simulation of thermograms with the following parameters: B_{LC} = 0.37, $K_{P,LC}$ = 1 782 and K = 0.096 for C_7A, ΔH_{VH} = 2 800 kJ/mol, B_G = 0.52

Computer Simulation

In many of the papers where the effects of various admixtures on the main phase transition temperature were studied it was assumed that these admixtures were only soluble in the liquid-crystalline phase L_α. In such a case, to interpret the experimental data of the effect of C_nA on the temperature of the main phase transition of DPPC, one could use a simple thermodynamic theory of the melting point depression of a solid due to admixtures. On using such a theory the dependences shown in Fig. 6 would suggest that the partition coefficient of C_nA between the aqueous and the phospholipid phase L_α increases up to $n = 8$ or 9 and, beginning at $n = 10$, it diminishes. However, the assumption of insolubility of C_nA in the gel phases does not appear to be correct in our case, probably like in other publications where the effect of amphiphilic admixtures on the temperature of the main transition of DPPC and other synthetic phospholipids is studied. Therefore, we used the results of Sturtevant[29] who proceeds from the assumption that the partition coefficient $K_{P,G}$ of amphiphilic admixtures between the gel phase of the lipid and the aqueous phase may be different from zero and different from the partition coefficient $K_{P,LC}$ for a liquid-crystalline lipid. On the assumption that a theory of ideal solutions can be used and that the peak width of the heat capacity $C_P = f(T)$ is composed of two additive parts, a van't Hoff broadening and a broadening due to the presence of an admixture, Sturtevant derived the equation

$$T_m^0 = \{1 + \boldsymbol{R}T_m^0 [(\frac{1}{\Delta H_{VH}^0}) \ln \frac{(1-\alpha)}{\alpha} - \frac{(1-K)}{\Delta H_{cal}^0} \ln x_{L,LC}]\}\ T \qquad (8)$$

for the relationship between temperature T (in K) and the extent of the phase transition α. Symbols ΔH_{cal}^0, ΔH_{VH}^0 and T_m^0 in Eq. (*8*) refer to a pure phospholipid phase without the admixture. The first term in the parentheses is the so-called van't Hoff broadening, the second term expresses the effect of the admixture, the molar fraction of which in the liquid-crystalline phase is $x_{A,LC} = 1 - x_{L,LC}$. The partition coefficient of the admixture between the liquid-crystalline phase of the lipid and the aqueous phase is given by

$$K_{P,LC(x)} = x_{A,LC} / x_{A,W} \qquad (9)$$

and the partition coefficient of the admixture between the lipid gel phase and the aqueous phase is given by

$$K_{P,G(x)} = x_{A,G} / x_{A,W} \ . \qquad (10)$$

The partition coefficient of the admixture between the gel and the liquid-crystalline phase of the lipid is given by

$$K = x_{A,G}/x_{A,LC} = K_{P,G(x)}/K_{P,LC(x)} \quad . \tag{11}$$

The molar fractions $x_{A,LC}$, $x_{A,G}$ and $x_{A,W}$ are defined as follows:

$$x_{A,LC} = n_{A,LC}/(n_{A,LC} + n_{L,LC}) \tag{12}$$

$$x_{A,G} = n_{A,G}/(n_{A,G} + n_{L,G}) \tag{13}$$

$$x_{A,W} = n_{A,W}/(n_{A,W} + n_W) \quad , \tag{14}$$

where n_A is the total number of moles of the admixture, n_L is the total number of moles of the lipid, n_W is the total number of moles of water, $n_{A,LC}$ is the number of moles of the admixture in the liquid-crystalline phase of the lipid, $n_{A,G}$ is the number of moles of the admixture in the gel phase of the lipid, $n_{A,W}$ is the number of moles of the admixture in the aqueous phase, $n_{L,LC} = \alpha n_L$ is the number of moles of the lipid in the liquid-crystalline phase and $n_{L,G} = (1 - \alpha)n_L$ is the number of moles of the lipid in the gel phase.

Occasionally the partition coefficients of the admixture are expressed not by the molar fractions but by the molar or mass concentrations of the admixtures in the phospholipid and the aqueous phases and are designated as K_P or $K_{P(m)}$, respectively. They can be interconverted as follows:

$$K_{P(x)} = (M_L/M_W)K_{P(m)} = (V_L/V_W)K_P \quad , \tag{15}$$

where M_L and M_W are the molar masses of the lipid and water, respectively, and, similarly, V_L and V_W are the corresponding molar volumes. If the partition coefficient between DPPC and water is examined, the use of the known values for M_L, M_W and V_L (ref.[30]) and V_M (ref.[31]) leads to the following conversion formula

$$K_{P(x)} = 40.6\, K_{P(m)} = 42.5\, K_P \quad . \tag{16}$$

Combination of (*9*) – (*14*) makes it possible to express $n_{A,LC}$ as the root of the cubic equation

$$A(n_{A,LC})^3 + B(n_{A,LC})^2 + Cn_{A,LC} + D = 0 \ , \qquad (17)$$

where the constants *A*, *B*, *C* and *D* are defined as follows:

$$A = (K_{P,LC(x)} - 1)(1 - K) \qquad (18)$$

$$B = (1 - K)[n_L\alpha(2K_{P,LC(x)} - 1) - n_A\,(K_{P,LC(x)} - 1) + n_W] + (K_{P,LC(x)} - 1)Kn_L \qquad (19)$$

$$C = n_L\alpha\{KK_{P,LC(x)}\,[n_A + (1 - \alpha)n_L] + n_W + n_A - n_A(2K_{P,LC(x)} - 1)\} \qquad (20)$$

$$D = -K_{P,LC(x)}n_An_L^2\alpha^2 \ . \qquad (21)$$

In view of the fact that $n_{A,LC}$ expresses the number of moles of the admixture in the liquid-crystalline phase of the lipid, we shall only take into account the positive solutions of Eq. (*17*). One can then calculate the $n_{A,LC}$ for every α and substitute it into Eq. (*12*). The $x_{A,LC}$ thus determined can be used via $x_{L,LC} = 1 - x_{A,LC}$ in Eq. (*8*) and the relationship between T and α can be calculated. If then the same procedure is used to determine T_2 and T_1 for the set of values $\alpha + \delta\alpha$, $\alpha - \delta\alpha$ one can calculate the molar heat capacity according to

$$C_P = 2\delta\alpha\ \Delta H_{cal}\,/(T_2 - T_1) \qquad (22)$$

which makes it possible to do computer simulation of the thermograms on the basis of parameters T_m^0, ΔH_{cal}^0, ΔH_{VH}^0, $K_{P,LC(x)}$ and K. Such simulations were performed with the aim of finding agreement between the experimental dependence in Fig. 6 and that from the simulated thermograms. In the simulations we calculated the molar heat capacity for 100 values of α in the interval 0 – 1, with $\delta\alpha = 0.005$. Examples of the simulated thermograms are shown in Figs 7 and 8. The effect of $K_{P,LC}$ on the thermograms is shown in Fig. 7. It is clear that an increase of $K_{P,LC}$ with the other parameters of the phase transition remaining constant, causes a decrease of T_m and a broadening and deformation of the endothermic peak. The effect of K on the position and shape of the thermogram can be observed in Fig. 8. If the other parameters are constant, the largest drop of T_m is observed for $K = 0$ ($K_{P,G} = 0$). With increasing K the simulated thermo-

grams approach more and more the thermogram of a pure lipid until for $K = 1$ ($K_{P,G} = K_{P,LC}$) they are identical. If K > 1 ($K_{P,G} > K_{P,LC}$) a rise of T_m may be observed. The decrease of T_m, as was found in the present experiments, is according to Sturtevant's theory caused by the fact that for C_nA holds $0 < K_{P,G} < K_{P,LC}$. At the same time we found that the value of ΔH_{VH} substantially affects the form of the thermogram even within the accuracy of the experimental values.

We shall now briefly describe the procedure for seeking the quantitative agreement between the experimental and the simulated thermogram for DPPC at c_{LA} = 0.103 mmol/dm^3 of the C_7A homologue. It is the relatively most effective of the several procedures we had tested. The experimental thermogram was first used to define four fundamental values: temperature T_A at which $C_P(T_A) = 0.5C_{P,max}$ from the end of lower temperature values than T_m, temperature T_B at which $C_P(T_B) = 0.5C_{P,max}$ from the end of higher temperature values than T_m, the T_m itself and the value of $C_{P,max}$ at T_m. By suitable steps we altered $K_{P,LC}$, K and ΔH_{VH} and sought such values as would fulfill the following conditions:

$$\delta C_{P,max} = | C_{P,max}^{sim} - C_{P,max} | \leq 10 \text{ J/mol K} \qquad (23)$$

$$\delta T_m = | T_m^{sim} - T_m | \leq 0.1 \text{ K} \qquad (24)$$

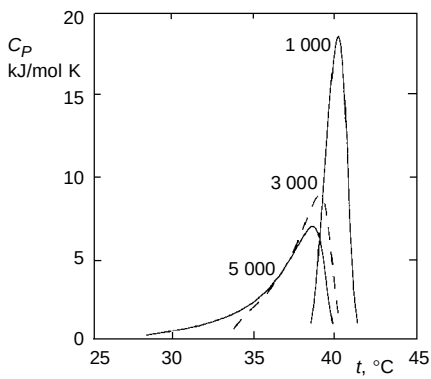

FIG. 7
Schematic representation of the effect of $K_{P,LC}$ on the shape of the simulated thermograms. The numerals at the individual thermograms stand for the value of $K_{P,LC}$ used in the simulation. Other parameters used for the stimulation were ΔH_{cal} = 30.5 kJ/mol, ΔH_{VH} = 3 500 kJ/mol, K = 0

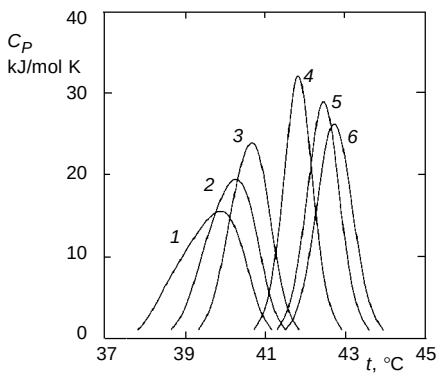

FIG. 8
Schematic representation of the effect of K on the shape of simulated thermograms. The values of K: *1* 0, *2* 0.2, *3* 0.4, *4* 1, *5* 1.4, *6* 1.6. The parameters used for the simulation were ΔH_{cal} = 30.5 kJ/mol, ΔH_{VH} = 3 500 kJ/mol, $K_{P,LC}$ = 1 288

$$\delta C_{P,A} = | C_P^{sim}(T_A) - C_P(T_A) | \leq 1.5 \text{ kJ/mol K} \quad (25)$$

$$\delta C_{P,B} = | C_P^{sim}(T_B) - C_P(T_B) | \leq 1.5 \text{ kJ/mol K} \ , \quad (26)$$

where the index sim stands for values obtained by simulation. The simulated thermograms served as the basis for determining the sum of squares of deviations from the experimental values

$$\sum \varepsilon^2 = (\delta T_m)^2 + (\delta C_{P,A})^2 + (\delta C_{P,B})^2 \ . \quad (27)$$

The relationships $\Sigma\varepsilon^2 = f(\log K_{P,LC})$ and $\Sigma\varepsilon^2 = f(\Delta H_{VH})$ from the results of simulations fulfilling conditions (*23*) – (*26*) for the individual values of K pass through a minimum which is the deeper the greater the value of K. For every K we determined the $K_{P,LC}$ value at which a minimum of $\Sigma\varepsilon^2$ is observed and plotted log $K_{P,LC}$ as a function of K, as shown in Fig. 9. From the relation $\Sigma\varepsilon^2 = f(\Delta H_{VH})$ every pair of K, $K_{P.LC}$ is amenable to deriving the corresponding value of ΔH_{VH} at which the value of $\Sigma\varepsilon^2$ has its minimum. Thermograms, simulated by using these data are compared with an experimental thermogram in Fig. 10. A fine agreement is seen both in the position and the intensity of the peak and relatively good agreement in the form of the curve for $C_P > 3$ kJ/mol.

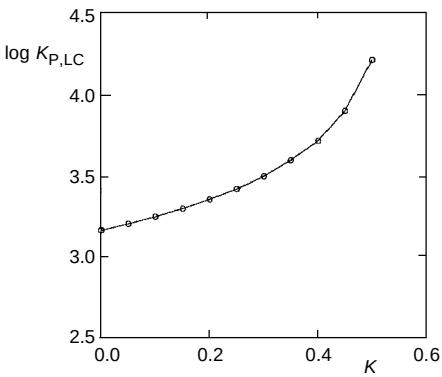

FIG. 9
Dependence of log $K_{P,LC}$ on K as the result of simulations according to conditions (*23*) – (*26*)

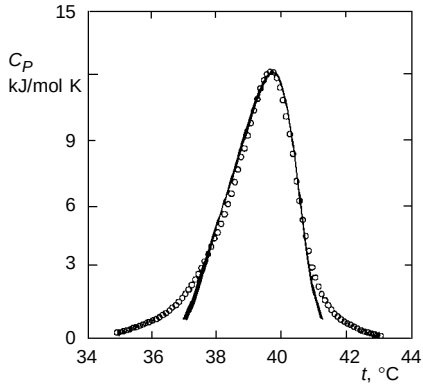

FIG. 10
Comparison of the experimental thermogram (C_7A, c_{LA} = 0.103 mmol/dm^3 in a system of DPPC–water, pH 6.2, shown by circles) with simulated thermograms (full lines) in the range of parameters of Fig. 9

For lower values of C_P the experimental and simulated values are quite different. Figure 10 indicates that thermograms simulated by using different input data for K, $K_{P,LC}$ and ΔH_{VH} differ less than any of these from the experimental one. On this basis one can conclude that Eq. (*8*) makes it possible to select such triplets of values of K, $K_{P,LC}$ and ΔH_{VH} that would keep the relation $C_P^{sim} = f(T)$ in a relatively good agreement between the simulated and the experimental result. However, it has not been possible to select a triplet that would best express the partition coefficients of the admixture between the lipid and aqueous phases.

Apparently one of the parameters K, $K_{P,LC(x)}$ or ΔH_{VH} must be known from an independent experiment before simulation. From the experimental point of view our work is closest to that in refs[32,33], where spectrophotometry was used for the determination of the partition coefficients of C_nA between the aqueous and the lipid phase, specifically unilamellar liposomes from egg-yolk phosphatidylcholine (EYPC), at pH 5 – 6. Under these conditions the phospholipid bilayer of EYPC liposomes is fluid. Absorption spectra of C_nA in the UV region exhibit two maxima, at $\nu_1 = 40\ 680\ cm^{-1}$ and $\nu_2 = 34\ 640\ cm^{-1}$. Since ref.[33] contains only a part of the results and ref.[32] is difficult to obtain we show here with the authors' consent some of their experimental results (Table III) as they are essential for computer simulation. If one assumes, in agreement with other experimental findings[34–38], that the increment of free energy of transfer of amphiphiles from the aqueous to the phospholipid phase is constant per methylene group of the hydrocarbon substituent in a given homologous series, the partition coefficient is defined by

$$\log K_{P,LC} = A_{LC} + B_{LC}\, n \qquad (28)$$

and the experimental findings of Table III permit the use of the least-squares method to obtain values of A_{LC} and B_{LC} and then, using Eq. (*28*), the calculation of $K_{P,LC}$ for any desired homologue. Using data obtained at the first maximum for the dependence of

TABLE III
Values of $K_{P,LC}$ (from the maximum at ν_1 and ν_2, respectively) for the C_nA homologues with different length of the alkoxy substituent n, taken from the papers of Hanus[27] and Balgavy et al.[28]

n	$K_{P,LC}$ (ν_1)	$K_{P,LC}$ (ν_2)
5	211.3	193.7
7	1 782	1 990
9	6 562	7 474

$K_{P,LC}$ on n we obtained $A_{LC} = 0.52$ and $B_{LC} = 0.37$; from the data obtained at the second maximum ν_2 then $A_{LC} = 0.38$ and $B_{LC} = 0.40$. For both values of the partition coefficient for C_7A shown in Table III we then selected suitable values for ΔH_{VH} and K and, using Eq. (*11*) we determined the partition coefficient for C_7A between the aqueous and the lipid phase in the gel state, $K_{P,G}$. It is assumed that even $K_{P,G}$ depends on the length of the alkoxy substituent n in a similar way as $K_{P,LC}$ does, viz.

$$\log K_{P,G} = A_G + B_G n \,. \qquad (29)$$

We then postulated that $B_G \geq B_{LC}$ and, therefore, in all further calculations, we altered B_G in suitable steps within the interval 0.37 – 0.64. Relationships (*28*) and (*29*) then were used to determine pairs of $K_{P,G}$ and $K_{P,LC}$ for the whole homologous series of C_nA. These data were then used for the simulation of thermograms according to Eq. (*8*) and for the evaluation of $\Delta T_m^{sim} = T_m^{sim} - T_m^0$ in dependence on the length of the alkoxy substituent n. This procedure was then repeated for different values of the slope of B_{LC} within the interval 0.37 – 0.40. All the dependences $\Delta T_m^{sim}/c_{LA} = f(n)$ calculated here were compared with the experimental values of Fig. 6. The best fit of the calculated values with the experimental ones was found for limiting B_{LC} values in the following two combinations: $B_{LC} = 0.37$, $K_{P,LC} = 1\,782$, $K = 0.096$ for C_7A, $\Delta H_{VH} = 2\,800$ kJ/mol, $B_G = 0.52$, and $B_{LC} = 0.40$, $K_{P,LC} = 1\,782$, $K = 0.096$ for C_7A, $\Delta H_{VH} = 2\,800$ kJ/mol, $B_G = 0.56$.

It may be seen that the decrease of efficiency of the $C_{10}A$ homologue to decrease the temperature of the main phase transition of DPPC in comparison with the shorter homologues C_8A and C_9A, as observed from the experimental dependence of $\Delta T_m/c_{LA} = f(n)$ in Fig. 6, is caused by an increase of slope B_G in Eq. (*29*) for the partition coefficient of C_nA homologues between the aqueous phase and the P_β gel phase of DPPC as compared with the slope B_{LC} for the liquid-crystalline phase L_α. The slopes B_{LC} and B_G were used to calculate the increment of free energy $\delta\Delta G^0$ for the transfer of one methylene group of the aliphatic chain of the admixture C_nA from the aqueous to the liquid-crystalline fluid or gel phase of DPPC. We found it to be between $-0.85\boldsymbol{R}T$ and $-0.92\boldsymbol{R}T$ for the fluid phase and between $-1.20\boldsymbol{R}T$ and $-1.29\boldsymbol{R}T$ for the gel phase. A qualitatively identical result was obtained by Lee[39] for the homologous series of 1-alkanols and by Inoue et al.[40] for the homologous series of *N*-alkyl-*N*,*N*,*N*-trimethylammonium bromides, interacting with DPPC. The interaction of the admixed molecules with long linear hydrocarbon substituents and DPPC bilayers is then analogous to the interaction of n-alkanes in bulk phase[41]: If the difference in the length of two alkanes is small (less than 6 methylene groups) they are soluble both in the liquid and in the solid phase; if the difference is larger they are soluble only in the liquid but not in the solid phase.

The values of the partition coefficients between the aqueous phase and a model biomembrane are often used as parameters in different correlations between biological efficiency and structure of the compounds under study. The model membrane is usually imitated by the n-octanol bulk-phase. However, both the present results and those of other authors[39–40] indicate that such a model can be misleading, as the observed biological effect is affected by changes in the equilibrium between domains of liquid-crystalline and gel phases of phospholipids in the target biomembrane. In this case, the biological effect is determined not by one partition coefficient of the biologically active compound but at least by two.

For instance, Lee[39,42] assumes a mechanism of anesthesia where the molecules of the anesthetics fluidize the annular gel-like layer of phospholipids around a sodium channel. On the one hand, the anesthetic concentration in this layer will depend on the ratio between total volume of the domains with properties of fluid liquid-crystalline phase and gel phase in the target membrane, on the other hand, its fluidization, i.e. the value of α of the anesthetic-induced isothermic phase transition in the annular lipid layer will be, at the same concentration of applied anesthetic, a nonlinear, quasiparabolic function of the length of the hydrocarbon chain in the homologous series. If Lee's hypothesis is correct the observed difference in the slopes B_G and B_{LC} could account for the quasiparabolic form of the local-anesthetic efficiency in dependence on the length of the substituent in the homologous series of tertiary amines that has been known for more than 60 years[43], as well as for the homologous series used in our earlier work[16].

It should be said in conclusion that the result of our simulations can only be taken for correct from a qualitative but not necessarily quantitative view. First of all, the values of $K_{P,LC}$ used in the simulations were obtained with another experimental model of the phospholipid bilayer (EYPC rather than DPPC used for the calorimetry). The accuracy of the simulations would also be enhanced by the knowledge of $\Delta t_m/c_{LA}$ for C_nA homologues with longer alkoxy substituents (n = 16 or 18). We have not been able to prepare them in sufficient quantities and purity for the calorimetric study. Likewise, the theoretical model used here as well as in other papers[29,34,35] is apparently a somewhat simplified, its principal shortcoming being in the postulate that the phase transitions between lyotropic liquid-crystalline mesophases can be described by an equilibrium according to Eq. (*2*) and hence the partition equilibria of the anesthetics by two partition coefficients $K_{P,LC}$ and $K_{P,G}$. Jörgensen et al.[21] carried out recently a Monte Carlo study of the admixture binding to lipid bilayers. On the assumption that the interaction of the admixture molecule shows more attraction toward lipid molecules in the liquid-crystalline phase as compared with a lipid containing all acyl chains in the all-*trans*-configuration, they computed that T_m decrease with increasing admixture concentration, the dependence of $C_P = f(T)$ is broadened and $C_{P,max}$ decreases, which is in agreement with our experimental results. However, the dependence of the admixture concentration in the lipid phase on temperature is qualitatively different from that based on Sturtevant's

model[29], which was used in the present work. The admixture concentration in the phase transition region first rises, reaches a maximum and then begins to decrease. This course is due to the lateral heterogeneity of the lipid bilayer in the region of phase transition as was mentioned above in connection with the pretransition. Its base consists in the existence of domains of liquid-crystalline lipid in the gel-phase matrix at temperatures below T_m and vice versa. The boundary between these domains is the site of structural defects and hence also of a higher concentration of the admixture which, in its turn, increases the surface area of the interface between the gel and the liquid-crystalline phase. The concentration of structural defects reaches its maximum in the region of the phase transition temperature. In this region one must then know not only the two partition coefficients $K_{P,LC}$ and $K_{P,G}$ but at least one other that would reflect the partition equilibria between the aqueous phase and the boundaries between the gel-phase and the liquid-crystalline-phase domains.

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