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SCANNING CALORIMETRIC STUDIES OF AQUEOUS DISPERSIONS OF BILAYERS MADE WITH CHOLESTEROL AND A PAIR OF POSITIONAL ISOMERS OF 3-*sn*-PHOSPHATIDYLCHOLINE

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Differential scanning calorimetric studies have been carried out on aqueous dispersions of cholesterol plus one of the pair of positional isomers, 1-arachidoyl-2-oleoylphosphatidylcholine (AOPC) or 1-oleoyl-2-arachidoylphosphatidylcholine (OAPC). There were some differences in the shapes of the endotherms obtained from the dispersions with the two positional isomers. These observations confirm the previous finding (Davis, P.J. and Keough, K.M.W. (1984) Biochemistry 22, 6334–6340) that positional isomers of unsaturated phosphatidylcholines may interact differently with cholesterol, at least in the gel state. The shapes of endotherms obtained here and in the previous study are consistent with the suggestion that the position of the unsaturated chain on the glycerol has a role in determining the nature of the phosphatidylcholine-cholesterol interaction. Certain features of the endotherms seen here and previously (op. cit.) suggest that factors such as effective chain depth in the bilayer or efficiency of chain packing in the bilayer may also influence this interaction.

The amount of cholesterol in different membranes varies from near zero to about 50 percent on a molar basis (for reviews, see for example, Refs. 1–4). Plasma membranes often contain high amounts of cholesterol whereas the membranes of subcellular organelles usually have less than 30 mol% (see Ref. 2). The cholesterol to phospholipid ratios of some abnormal cells and their membranes are altered in comparison to those of normal cells (see Ref. 4). Pulmonary surfactant contains up to about 15 mol% cholesterol (see Refs. 5 and 6).

The concept that cholesterol acts as a modulator of the order and of the motion of the hydrocarbon chains of membranes [7] has been supported by a large body of evidence (see Refs. 1–3 and 8). Aspects of sterol structure which influence the physical properties of membranes have been studied (for a review, see for example, Ref. 9). Also, work has been done on the effect of changing the headgroup or the chain length on phospholipid-cholesterol interactions (see Ref. 2), and some evidence for preferential association between cholesterol and some classes of lipids exists.

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Abbreviations: AOPC, 1-arachidoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; OAPC, 1-oleoyl-2-arachidoyl-*sn*-glycero-3-phosphocholine; OSPC, 1-oleoyl-2-stearoyl-*sn*-glycero-3-phosphocholine; SOPC, 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; T_c, gel to liquid-crystalline transition temperature; T_{max}, the temperature of maximum heat flow in an observed calorimetric event.

In a recent differential scanning calorimetric study we found that the endotherms of gel to liquid-crystalline transitions were different for membranes composed of cholesterol plus 1-stearoyl-2-oleoylphosphatidylcholine (SOPC) in comparison to those for membranes made with its positional isomer, 1-oleoyl-2-stearoylphosphatidylcholine (OSPC) [10]. These observations were interpreted as being suggestive of a difference in the nature of the PC/cholesterol association between the two isomers [10]. Such a difference could arise because of a specific orientation between cholesterol and each individual PC, such that in the case of the two isomers either a saturated or an unsaturated chain was adjacent to a given face of a cholesterol molecule [11]. The packing of chains in the gel state appears to vary with each pure positional isomer (see Refs. 12 and 13), and such differences in packing between isomers could also influence the nature of the cholesterol-lipid associations [10].

In order to determine if the behaviour observed with SOPC or OSPC plus cholesterol was unique to that pair of isomers and to shed further light on the nature of the interaction of cholesterol with positional isomers, we have studied the calorimetric properties of dispersions of cholesterol plus each of another pair of isomers, 1-arachidoyl-2-oleoyl-PC (AOPC) or 1-oleoyl-2-arachidoyl-PC (OAPC). For comparison, thermograms of cholesterol plus diarachidoyl-PC (DAPC) were also obtained. The properties of the thermograms can be interpreted in a manner which is consistent with both chain position and chain packing influencing the nature of the phosphatidylcholine-cholesterol interaction.

Materials and Methods

Materials. Fatty acids and cholesterol (> 99% pure) were obtained from Sigma Chemical Company, St. Louis, MO. Fatty acids were found to be pure (> 99%) by gas-liquid chromatography [11, 14], and were used without further purification. Cholesterol was re-crystallized twice from 95% ethanol, vacuum-dried over P_2O_5 for 16 h, and stored at −20°C. Other materials and reagents were obtained and treated as described previously [10,12,13].

AOPC, DAPC, DOPC, and OAPC were made by methods described previously [10]. OAPC and AOPC were crystallized from hexane/methanol (98 : 2, v/v) [10]. Thin layer chromatography and phosphorus assay indicated that these lipids contained 0–1.4% of a component with the mobility of 1,3-phosphatidylcholine. In addition, AOPC had a second minor component (0.8% of total P) with mobility slightly greater than that of 1,3-phosphatidylcholine. OAPC contained less than 1% of its positional isomer (AOPC) while AOPC had 6% OAPC [12].

Calorimetry. Aqueous dispersions of lipids at 33% (w/w) were prepared as described before [10,12]. Differential scanning calorimetry was performed using a Perkin-Elmer DSC-2 operated at 5 deg C/min in a manner described previously [10,12]. Experimental thermograms were normalized per mole of lipid phosphorus in the way outlined before [10]. The normalized endotherms were analyzed for component parts using a computer program for deconvolution which has also been described previously [10,15].

Results

Fig. 1 shows the normalized endotherms obtained for aqueous dispersions of mixtures of cholesterol with either AOPC, OAPC or DAPC. At cholesterol concentrations up to 17 mol% the endotherms for AOPC/cholesterol were skewed to high temperature and well-described by two components, one narrow (at low temperature) and one broad (at high temperature). Endotherms for OAPC/cholesterol were more symmetric than those for AOPC/cholesterol, although resolution of the former endotherms into two components could be done. Even then, OAPC/cholesterol endotherms had one major and a variable minor component. The endotherms obtained from AOPC/cholesterol were different from those obtained for the two other mixtures in that they had considerable high-temperature asymmetry. Those for DAPC/cholesterol and OAPC/cholesterol were fairly symmetric, although DAPC/cholesterol displayed some slight low-temperature asymmetry.

Table I gives a summary of values of various parameters obtained for the components obtained

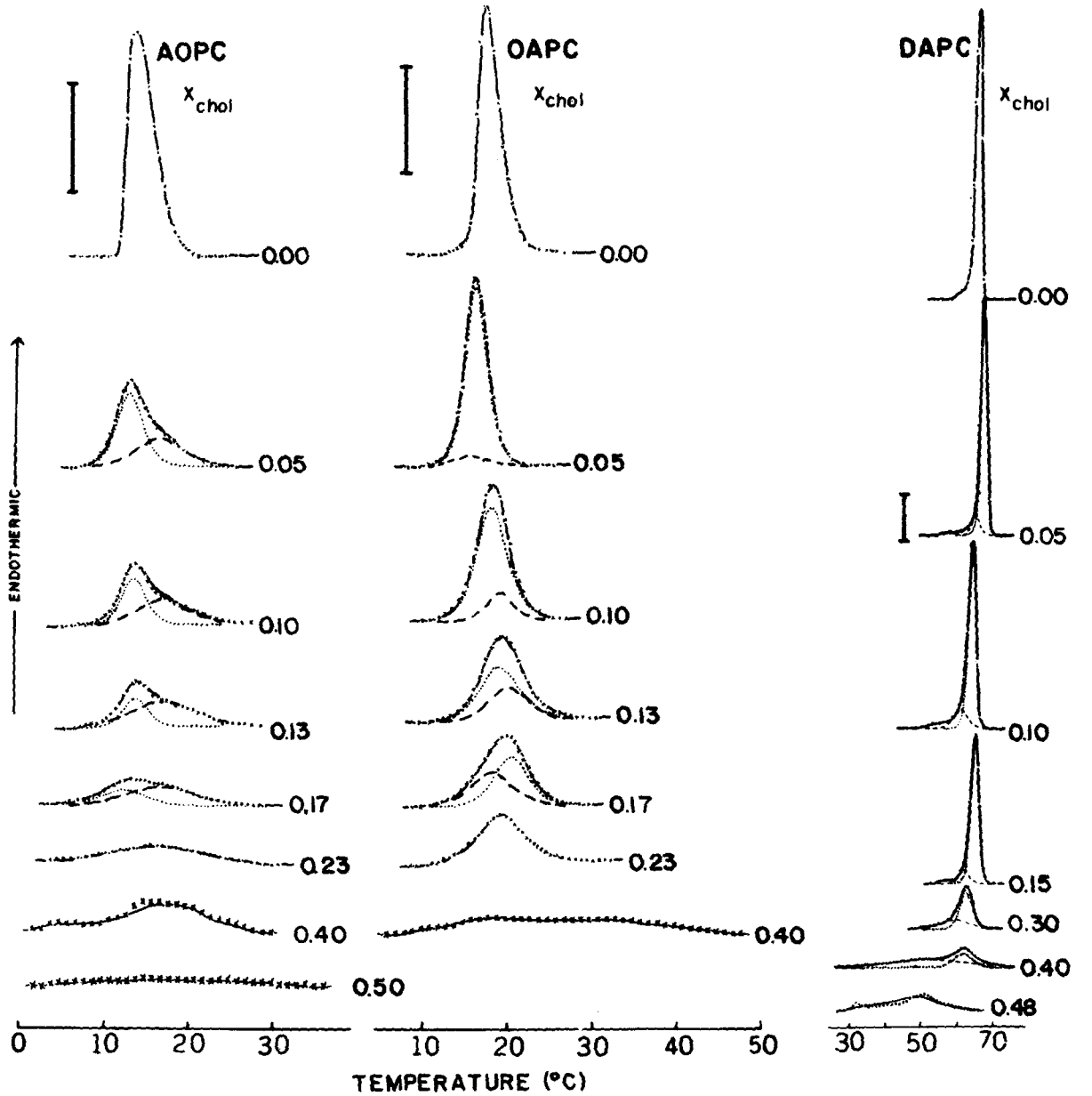

Fig. 1. Normalized endotherms of AOPC/cholesterol, OAPC/cholesterol, and DAPC/cholesterol. (×) Points from normalized experimental endotherms; (———) composite curves from the curve deconvolution procedure; (- - - - - -) broad and (· · · · · ·) narrow components from curve deconvolution. Scales represent 1 kcal·mol^{-1}·deg^{-1} C.

with the deconvolution program. Fig. 2 shows, as a function of cholesterol concentration, the total enthalpy of the transitions of AOPC/cholesterol and OAPC/cholesterol (Fig. 2A), the enthalpy of the narrow components (Fig. 2C) and the enthalpy of the broad components (Fig. 2D). Also included are the relative contributions of the narrow component to the total enthalpy of each individual endotherm (Fig. 2B). At 40 mol% cholesterol there was no observable transition for AOPC/cholesterol but one was discernible for OAPC/cholesterol (Figs. 1 and 2A). When the relative contribution of

TABLE I

VALUES FOR PARAMETERS OF DECONVOLUTED CURVES FOR MIXTURES OF VARIOUS PHOSPHATIDYLCHOLINES AND CHOLESTEROL

PC	X_{chol} [a]	SD [b] (cal·mol^{-1}·deg^{-1})	Narrow component					Broad component				
			T_{max} [c] (°C)	C_{max}^{ex} [d] (cal·mol^{-1}·deg.$^{-1}$)	$\Delta T_{1/2}$ [e] (deg.)	ΔH_{cal} [f] (cal·mol^{-1})	$\Delta H_{van't\ Hoff}$ [g] (cal·mol^{-1})	T_{max} [c] (°C)	C_{max}^{ex} [d] (cal·mol^{-1} deg.$^{-1}$)	$\Delta T_{1/2}$ [e] (deg.)	ΔH_{cal} [f] (cal·mol^{-1})	$\Delta H_{van't\ Hoff}$ [g] (cal·mol^{-1})
AOPC	0.05	27.3	12.2	618	3.3	$2.58 \cdot 10^3$	$1.55 \cdot 10^5$	15.2	306	6.3	$2.23 \cdot 10^3$	$9.09 \cdot 10^4$
	0.05	25.2	12.5	682	3.3	$2.78 \cdot 10^3$	$1.59 \cdot 10^5$	16.1	273	6.0	$1.94 \cdot 10^3$	$9.36 \cdot 10^4$
	0.10	12.2	13.1	428	3.0	$1.67 \cdot 10^3$	$1.67 \cdot 10^5$	16.4	227	7.0	$1.87 \cdot 10^3$	$8.09 \cdot 10^4$
	0.13	12.3	13.8	358	3.5	$1.48 \cdot 10^3$	$1.58 \cdot 10^5$	17.4	217	10.8	$2.65 \cdot 10^3$	$5.51 \cdot 10^4$
	0.13	9.0	13.1	273	3.0	$1.07 \cdot 10^3$	$1.67 \cdot 10^5$	16.3	231	9.0	$2.37 \cdot 10^3$	$6.49 \cdot 10^4$
	0.17	5.8	11.8	138	5.8	$0.92 \cdot 10^3$	$9.69 \cdot 10^4$	16.1	173	9.3	$1.83 \cdot 10^3$	$6.29 \cdot 10^4$
OAPC	0.05	30.9	16.2	1529	3.3	$5.81 \cdot 10^3$	$1.76 \cdot 10^5$	13.5	219	3.3	$0.83 \cdot 10^3$	$1.73 \cdot 10^5$
	0.05	35.2	13.8	1656	3.0	$5.60 \cdot 10^3$	$1.91 \cdot 10^5$	12.9	95	4.3	$0.47 \cdot 10^3$	$1.31 \cdot 10^5$
	0.10	38.7	15.4	1021	3.8	$4.72 \cdot 10^3$	$1.43 \cdot 10^5$	15.5	247	5.3	$1.75 \cdot 10^3$	$1.18 \cdot 10^5$
	0.13	23.7	15.1	494	5.2	$2.96 \cdot 10^3$	$1.11 \cdot 10^5$	16.1	779	5.3	$1.75 \cdot 10^3$	$1.18 \cdot 10^5$
	0.13	14.6	16.4	774	4.5	$4.03 \cdot 10^3$	$1.28 \cdot 10^5$	19.1	485	3.0	$1.56 \cdot 10^3$	$2.14 \cdot 10^5$
	0.17	14.0	17.2	449	4.5	$2.39 \cdot 10^3$	$1.26 \cdot 10^5$	14.8	318	5.8	$2.02 \cdot 10^3$	$1.04 \cdot 10^5$
DAPC	0.05	194	65.4	5109	2.0	$1.12 \cdot 10^4$	$4.16 \cdot 10^5$	63.0	457	2.5	$1.36 \cdot 10^3$	$3.03 \cdot 10^5$
	0.10	156	62.1	4115	2.3	$1.11 \cdot 10^4$	$3.31 \cdot 10^5$	59.1	412	4.3	$2.03 \cdot 10^3$	$1.78 \cdot 10^5$
	0.15	107	63.2	3316	2.5	$9.33 \cdot 10^3$	$3.21 \cdot 10^5$	60.2	301	3.0	$1.10 \cdot 10^3$	$2.63 \cdot 10^5$
	0.30	30.5	60.8	828	3.8	$3.43 \cdot 10^3$	$2.14 \cdot 10^5$	57.4	194	9.5	$2.10 \cdot 10^3$	$8.03 \cdot 10^4$
	0.40	24.5	62.7	298	3.3	$2.13 \cdot 10^3$	$1.25 \cdot 10^5$	53.1	178	13.3	$5.34 \cdot 10^3$	$2.82 \cdot 10^4$

[a] Mole fraction of cholesterol.
[b] Standard deviation of the residuals of excess heat capacity between the observed data and calculated total endotherm.
[c] The temperature of maximum excess heat capacity calculated for the component curve.
[d] The maximum excess heat capacity calculated for the component curve.
[e] The width at one half C_{max}^{ex} for each deconvoluted curve component.
[f] The calculated calorimetric enthalpy of the deconvoluted component.
[g] The calculated van't Hoff enthalpy of the deconvoluted component.

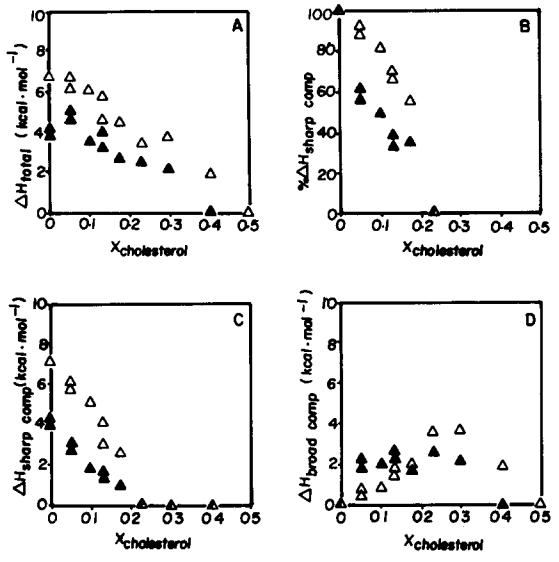

Fig. 2. Enthalpies associated with transition of AOPC/cholesterol (▲) and OAPC/cholesterol (△). (A) Total transition enthalpies; (B) percentage of the total enthalpy of each individual endotherm which was contributed by the narrow component (C) absolute enthalpy of the narrow component; (D) absolute enthalpy of the broad components.

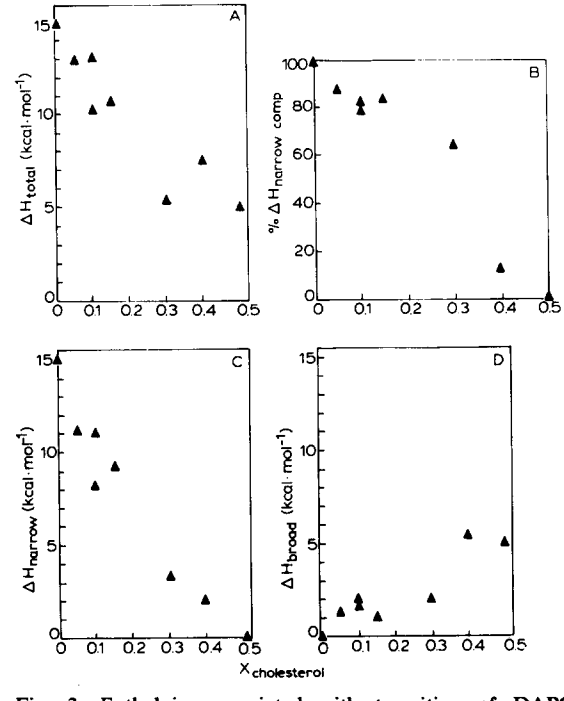

Fig. 3. Enthalpies associated with transition of DAPC/cholesterol. (A) Total enthalpy; (B) percentage of the total enthalpy of each individual endotherm which was contributed by the narrow component; (C) absolute enthalpy of the narrow component; (D) absolute enthalpy of the broad component.

the narrow component to the total enthalpy of each individual endotherm is calculated, that contribution decreases more rapidly in the case of AOPC/cholesterol than with OAPC/cholesterol (Fig. 2B).

Fig. 3 shows data on the enthalpy of the mixtures of DAPC and cholesterol. Data for DOPC/cholesterol are published in Ref. 10, as are data for SOPC/cholesterol and OSPC/cholesterol. DAPC and OAPC are similar when mixed with cholesterol in that the proportion of the total enthalpy of any endotherm contributed by the narrow component is relatively high (Figs. 3B and 2B) in comparison to the contribution by the narrow component to the total enthalpy in AOPC/cholesterol (Fig. 2B).

The temperatures of maximum heat flow for the components of the endotherms were nearly independent of cholesterol concentration for both the mixed-acid lipids. There was a decrease in the maximum temperature for the broad component of DAPC/cholesterol. This latter property was like that seen for DOPC/cholesterol.

Discussion

The absolute enthalpies of pure AOPC and pure OAPC are low considering the length of the chains. We have not found any analytical difference by thin-layer chromatography, gas-liquid chromatography or positional analysis which would suggest substantial contamination of either lipid. The transition temperatures of AOPC (T_c = 11.9°C, T_{max} = 12.2°C) and OAPC (T_c = 15.9°C, T_{max} = 16.3°C) followed the trend seen in other mixed-acid PC; that is, the isomer with the 1-long-2-short configuration had the lower transition temperature [12,13]. The enthalpies of other mixed-acid PC containing unsaturated chains were also relatively low considering their chain lengths [13]. The patterns of the relative enthalpies exhibited by AOPC and OAPC were consistent with those seen before [13] in that the 1-long-2-short isomer had the lower enthalpy.

The shapes of the endotherms for AOPC/cholesterol are not the same as those for OAPC/cholesterol, implying some difference in the association between cholesterol and the two isomers, at least in the gel state. The shapes of the endotherms for AOPC/cholesterol (Fig. 1) and SOPC/

cholesterol (Fig. 1 of Ref. 10) were similar, as were those from OAPC/cholesterol (Fig. 1) and OSPC/cholesterol (Fig. 1 of Ref. 10). The former group of endotherms were skewed to high temperature and readily resolvable into two components. Those in the latter group were almost symmetrical, were resolvable into two components, but could be described almost as well by one component. This pattern is consistent with the interpretation that the position of the unsaturated chain on the PC influences the interaction between PC and cholesterol [11]. Such a difference resulting from the interaction of the different positional isomers with cholesterol may be related to the observations that the membranes of some mycoplasmas [16,17] and some hepatomas [18–20] have high cholesterol contents and high contents of phospholipids with unsaturated chains in the *sn*-1 position of glycerol.

While there are overall similarities between the endotherms for SOPC/cholesterol [10] and AOPC/cholesterol, and between those for OSPC/cholesterol [10] and those for OAPC/cholesterol, there are some quantitative differences between the endotherms also. These are in the rates at which the endotherms broaden as a function of cholesterol concentration, the relative proportions of the enthalpies contributed by narrow components, and the ability to discern a transition at higher cholesterol concentration in OAPC than in OSPC (compare Figs. 1–3 of Ref. 10 and Figs. 1 and 2 of this paper). These small but potentially significant differences suggest that, in addition to the position of the saturated and unsaturated chains, the absolute chain lengths (see Refs. 10 and 21–24) and the relative difference in chain length and effective bilayer penetration (see Refs. 10 and 12–13) may also influence the shape of the endotherms.

The results obtained here, and those observed for mixtures of SOPC or OSPC plus cholesterol [10], suggest that the position of the unsaturated chain on the glycerol backbone of a phosphatidylcholine may have an influence on the nature of the PC-cholesterol interaction, at least in the gel state. The potential effectiveness of packing of the lecithin chains in the bilayer may also serve to modulate the interaction between the phospholipid and cholesterol.

Acknowledgments

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References

1 Demel, R.A. and De Kruijff, B. (1976) Biochim. Biophys. Acta 457, 109–132
2 Green, C. (1977) Int. Rev. Biochem. 14, 101–152
3 Headon, D.R., Barrett, E.J., Joyce, N.M. and O'Flaherty, J. (1977) Mol. Cell. Biochem. 17, 117–123
4 Chen, H.W., Kandutsch, A.A. and Heiniger, H.J. (1978) Prog. Exp. Tumor. Res. 22, 275–316
5 King, R.J. and Clements, J.A. (1972) Am. J. Physiol. 223, 715–726
6 Metcalfe, I.L., Enhorning, G. and Possmayer, F. (1980) J. Appl. Physiol.: Respir. Environ. Exercise Physiol. 49, 34–41
7 Ladbrooke, B.D., Williams, R.M. and Chapman, D. (1968) Biochim. Biophys. Acta 150, 333–340
8 Phillips, M.C. (1972) Prog. Surf. Membrane Sci. 5, 139–221
9 Block, K.E. (1983) CRC Crit. Rev. Biochem. 14, 47–92
10 Davis, P.J. and Keough, K.M.W. (1984) Biochemistry 22, 6334–6340
11 Huang, C.-H. (1977) Lipids 12, 348–356
12 Keough, K.M.W. and Davis, P.J. (1979) Biochemistry 18, 1453–1459
13 Davis, P.J., Fleming, B.D., Coolbear, K.P. and Keough, K.M.W. (1981) Biochemistry 20, 3633–3636
14 Thompson, W. (1969) Biochim. Biophys. Acta 187, 150–153
15 Spink, C.H., Müller, K. and Sturtevant, J.M. (1982) Biochemistry 21, 6598–6605
16 Rottem, S. and Markowitz, O. (1979) Biochemistry 18, 2930–2935
17 Rottem, S. and Markowitz, O. (1979) FEBS Lett. 107, 379–382
18 Dyatlovitskaya, E.V., Yanchevskaya, G.V. and Bergelson, L.D. (1974) Chem. Phys. Lipids 12, 132–149
19 Dyatlovitskaya, E.V., Timofeeva, N.G., Gor'Kova, N.P. and Bergelson, L.D. (1975) Biokhimiya 40, 1315–1319
20 Van Hoeven, R.P. and Emmelot, P. (1972) J. Membrane Biol. 9, 105–126
21 Mabrey, S., Mateo, P.L. and Sturtevant, J.M. (1978) Biochemistry 17, 2464–2468
22 Gershfeld, N.L. (1978) Biophys. J. 22, 469–488
23 Lundberg, B., Svens, E. and Ekman, S. (1978) Chem. Phys. Lipids 22, 285–292
24 McIntosh, T.J. (1978) Biochim. Biophys. Acta 513, 43–58