Biochimica et Biophysica Acta, 770 (1984) 7–14
Elsevier

BBA 71953

TEMPERATURE ACCLIMATION AND PHOSPHOLIPID PHASE TRANSITION IN HYPOTHALAMIC MEMBRANE PHOSPHOLIPIDS OF GARDEN LIZARD, *CALOTES VERSICOLOR*

G. DURAIRAJ and I. VIJAYAKUMAR

Department of Zoology, University of Madras, Madras-600 005 (India)

(Received July 7th, 1983)
(Revised manuscript received October 3rd, 1983)

Key words: Temperature acclimation; Phospholipid membrane; Phase transition; Differential scanning calorimetry; Phospholipid composition; Hypothalamus; (Lizard brain)

Garden lizards, *Calotes versicolor*, were acclimated to three different temperatures, i.e., 16°C, 26°C and 36°C for a period of 30 days in 'walk-in-environmental chambers'. The phospholipid profile and fatty acid pattern were analysed in the hypothalamus and brain of the acclimated animals. Hypothalamic and brain membrane phospholipids were prepared and their phase-transition temperatures were recorded using differential scanning calorimetry. Acclimation temperature, phospholipid composition, fatty acids of these phospholipids and the physical state and fluidity of the specific model membranes of hypothalamus (and brain) are intimately inter-related. Evidence is presented for the first time to show a possible correlation between acclimation temperature and phase-transition temperature of hypothalamic phospholipid membrane. A direct physico-chemical basis is suggested for the thermoregulatory process of hypothalamus leading to a better understanding of our knowledge on the origin of thermoregulation.

Introduction

Phospholipids, when dispersed in excess water at physiological temperatures, become arranged in lamellar arrays [1]. Studies utilising a variety of physical techniques have confirmed that these lamella consist of phospholipid bilayers separated by water-filled spaces [2]. A unique feature of this protein-free lipid bilayers is their ability to undergo a reversible thermotropic phase transition from a fluid state at high temperature to a crystalline state at low temperature. These order-disorder transitions in phospholipid bilayers parallel those in biomembranes. Phospholipids in water, therefore, provide a model system that is more relevant to real behaviour of natural membranes [3].

The phase transition of a phospholipid bilayer is an example of a two-dimensional co-operative transition. The phase transition characteristics, such as temperature and enthalpy of the transition, are dependent on the length and degree of unsaturation of the hydrocarbon chains, on the nature of phospholipid head group, on the presence or absence of water and on the ionic strength of the surrounding medium [4,5].

Differential scanning calorimetry has been applied to the study of the endothermic phase transition from a gel to the liquid-crystal state of simple-model biomembranes [6,7]. The first demonstration of the order-disorder transition in membranes of *Acholeplasma laidlawii* by calorimetry was made by Steim [8], which was later extended by other investigators [9–15].

Many such investigators have suggested that the lipids in biological membranes must exist in liquid-crystalline state for the cell membranes to

function normally [1,2]. Recent studies support the idea that the liquid-crystalline state is necessary to support growth, membrane transport [16], assembly and transport of proteins [17] and specific activity of membrane associated enzymes [18]. Although the hypothalamus has been recognized as the biological thermostat which senses changes in environmental temperature and processes the information for physiological thermoregulatory mechanisms by switching 'on' or 'off' specific circuits of neural systems, there is no information on the fluidity of phospholipid membrane of hypothalamus supported by phase transition study. To fill this gap, an attempt has been made to study the phospholipid phase transition of hypothalamic membranes, the phospholipid profile and their fatty acid composition in an experimental model system using lizards. As lizards (reptiles) are the most advanced ectotherms which show variations of body temperature depending on the changes in ambient temperature (unlike homeotherms) with some possible behavioural, but no physiological thermoregulation, the study could provide some real insight into our understanding of origin of thermoregulation.

Methods and Materials

Laboratory acclimation of animals

Garden lizards, *Calotes versicolor,* were acclimated in the laboratory at different temperatures (i.e., 16°C, 26°C and 36°C) in 'walk-in-environmental chambers' for a period of 30 days. Animals were maintained on termites as feed.

Lipid extraction, purification, phospholipid separation and estimation

After temperature acclimation, animals were decapitated, their hypothalamus and brains were surgically removed. From the samples of hypothalamus and brain, lipid was extracted by the procedure of Folch et al. [19]. All lipid extracts were evaporated to dryness under nitrogen. From the purified lipid samples, the total phospholipid fraction was separated on a silicic acid column following the method of Rouser et al. [20]. The extracted phospholipid was estimated gravimetrically. Approx. 1 g of silicic acid was used for an expected 25 mg of phospholipid.

The individual phospholipids were separated by TLC, using the solvent system chloroform/methanol/20% aqueous ammonia (65 : 25 : 5, v/v). The phospholipids were located using iodine vapour and estimated after perchloric acid (72%) digestion of the phospholipid spots [21,22].

Fatty acid analysis and quantification

Fatty acid methyl esters were prepared by transesterification using 14% BF_3-methanol [23] and separated in TLC plates [24]. The fatty acid methyl esters were dissolved in known volume of hexane and analysed using gas-liquid chromatography as described by Durairaj and Martin [25]. The conditions of separation of esters included a $8 \times 1/8$ inches copper column packed with 15% diethylene glycol succinate (DEGS) on Chromosorb AW operated at an oven temperature of 185°C, injection port and flame ionization detector temperature of 200°C; dry nitrogen as a carrier gas and heptadecanoic acid as an internal standard were used. From the relative retention-time values and peak height the relative area under each peak was computed making the necessary corrections as suggested by Brandt and Lands [26]. The area under each peak was taken as a measure of the quantity of that particular fatty acid [27]. The values were expressed as moles percent. In all the experiments, statistical significance between means was assessed using Student-Newman-Keul's (SNK) test [28].

Differential scanning calorimetry

The phase-transition temperatures of the extracted phospholipid were determined using a differential scanning calorimeter (Perkin-Elmer DSC II). All samples were run in Perkin-Elmer 15 λ (15 μl) volatile sample pans. Known quantities of phospholipid samples (5 ± 0.005 mg) taken in sample pans were hydrated (by addition of five volumes of water), sealed and their phase transition temperatures were recorded using DSC operated at 10°C/min with full scale range of 1 mcal/s, 5 mcal/s and 10 mcal/s. Stearic acid was used as a calibrant. An empty sealed pan was used as the control.

Results

Phospholipid composition

The effect of thermal acclimation on the percentage distribution of phospholipids in the hypothalamus and brain is presented in Tables I and II. The major phospholipid groups of both hypothalamus and brain are phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidic acid (PA), cardiolipin (CL) and sphingomyelin (Sph). At lower acclimation temperature (16°C), the percentage composition of a low melting point lipid, cardiolipin, increased 5.5-fold in hypothalamus and nearly 3-fold in brain, when compared to higher acclimation temperatures. Although PE and PI (with melting points less than 200°C) did not increase with increase in acclimation temperature, the phospholipids of high melting points (above 200°C) did increase at high (36°C) acclimation temperature. For instance, there was about 27–28% increase of PC (melting point 230°C) in hypothalamus and brain during hot (36°C) acclimation when compared with cold (16°C) acclimation. Another high melting point lipid, sphingomyelin, (melting point 210°C) increased by 3-fold both in hypothalamus and brain at 36°C acclimation when compared with 16°C allimation. There was no marked change during cold or hot acclimation in the percentage distribution of PS (high melting point) or PA (low melting point).

TABLE I

THE EFFECT OF THERMAL ACCLIMATION ON THE PERCENT DISTRIBUTION OF PHOSPHOLIPIDS IN HYPOTHALAMUS OF *C. VERSICOLOR*

Figures are presented as means ± S.E. CL, cardiolipin; Sph, sphingomyelin.

Phospholipid species	Distribution (%) at acclimation temperature		
	16°C	26°C	36°C
PC	30.218 ± 0.632	36.026 ± 0.318	42.042 ± 0.396
PE	29.709 ± 0.446	23.783 ± 0.346	19.273 ± 0.272
PI	19.932 ± 0.199	16.663 ± 0.524	11.290 ± 0.253
PS	6.311 ± 0.137 [a]	6.032 ± 0.268 [a]	6.344 ± 0.140 [a]
PA	5.928 ± 0.145 [b]	6.338 ± 0.280 [b]	6.930 ± 0.134 [b]
CL	4.323 ± 0.151	2.199 ± 0.208	0.808 ± 0.163
Sph	3.999 ± 0.197	8.266 ± 0.242	12.493 ± 0.233

[a] and [b], not significantly different at the 0.05 level of probability (SNK test).

TABLE II

THE EFFECT OF THERMAL ACCLIMATION ON THE PERCENT DISTRIBUTION OF PHOSPHOLIPIDS IN THE BRAIN OF *C. VERSICOLOR*

Figures are presented as means ± S.E. CL, cardiolipin; Sph, sphingomyelin.

Phospholipid species	Distribution (%) at acclimation temperature		
	16°C	26°C	36°C
PE	31.632 ± 0.510	26.335 ± 0.516	19.565 ± 0.270
PC	30.455 ± 0.741	38.199 ± 0.406	42.846 ± 0.357
PI	16.470 ± 0.260	13.820 ± 0.539	9.967 ± 0.306
PS	6.979 ± 0.125 [a]	6.997 ± 0.307 [a]	7.079 ± 0.128 [a]
PA	5.880 ± 0.152 [b]	5.679 ± 0.255 [b]	5.891 ± 0.180 [b]
CL	4.727 ± 0.172	2.864 ± 0.215	1.685 ± 0.149
Sph	3.951 ± 0.180	7.517 ± 0.264	11.817 ± 0.212

[a] and [b], not significantly different at the 0.05 level of probablity (SNK test).

Fatty acid composition

The effect of thermal acclimation on the percentage distribution of fatty acids in total phospholipid of hypothalamus is presented in Table III and of brain in Table IV. The major fatty acids found in the hypothalamus are 16:0, 18:0 and 18:2. The major fatty acids in brain are 16:0, 18:0, 18:1, 18:2 and 22:6. A general tendency of a higher degree of saturation at high acclimation temperature and a higher degree of unsaturation at low acclimation temperature was noticed. In the animals acclimated at 36°C, the increase in saturation of fatty acids in phospholipid was 2.8-fold in hypothalamus and 1.8-fold in brain. Correspondingly, the $n-9$ fatty acids decreased by 60% in hypothalamus and 30% in brain; the $n-6$ fatty acids decreased by 60% in hypothalamus and 55% in brain; and $n-3$ fatty acids decreased by 40% both in hypothalamus and brain. The increase in unsaturation index at 16°C is indicative of an increase in the ratio of enoic to anoic fatty acids.

Lipid phase transition

The effect of thermal acclimation on the phase transition temperatures of membrane phospholipids of hypothalamus and brain is given in Tables V and VI and Figs. 1 and 2.

The large transition centered at 0°C is due to

TABLE III

THE EFFECT OF THERMAL ACCLIMATION ON THE PERCENT DISTRIBUTION OF FATTY ACIDS IN TOTAL PHOSPHOLIPID OF HYPOTHALAMUS OF *C. VERSICOLOR*

Figures are presented as means ± S.E.

Fatty acids		Distribution (%) at acclimation temperature		
		16°C	26°C	36°C
Saturated				
14:0		0.43 ± 0.01 [a]	0.43 ± 0.006 [a]	0.44 ± 0.01 [a]
16:0		15.36 ± 0.40	26.55 ± 0.54	34.62 ± 0.31
18:0		7.64 ± 0.36	14.84 ± 0.64	22.49 ± 0.27
20:0		1.07 ± 0.09	3.34 ± 0.31	9.89 ± 0.38
Unsaturated				
Monounsaturated				
$n-9$	16:1	10.51 ± 0.47	7.02 ± 0.24	3.09 ± 0.14
	18:1	23.49 ± 0.39	14.87 ± 0.31	9.13 ± 0.42
	20:1	1.37 ± 0.16 [b]	1.58 ± 0.23 [b]	1.86 ± 0.06 [b]
Polyunsaturated				
$n-6$	18:2	14.63 ± 0.30	10.98 ± 0.16	6.00 ± 0.08
	20:2	0.67 ± 0.20 [c]	0.53 ± 0.14 [c]	0.77 ± 0.07 [c]
	20:3	0.78 ± 0.20 [d]	0.88 ± 0.10 [d]	0.72 ± 0.03 [d]
	20:4	1.12 ± 0.15 [e]	1.01 ± 0.11 [e]	0.94 ± 0.03 [e]
	22:4	2.38 ± 0.16	1.76 ± 0.06	0.70 ± 0.07
	22:5	4.72 ± 0.18	2.84 ± 0.09	0.69 ± 0.02
$n-3$	18:3	2.74 ± 0.51	1.74 ± 0.34	0.99 ± 0.02
	20:5	2.35 ± 0.35 [f]	2.94 ± 0.03 [f]	2.00 ± 0.06 [f]
	22:6	8.35 ± 0.21	7.68 ± 0.43	6.64 ± 0.04

[a–f], not significantly different at the 0.05 level of probability (SNK test).

	Acclimation temperature		
	16°C	26°C	36°C
Ratio $\left(\frac{\%\text{ unsaturated fatty acids}}{\%\text{ saturated fatty acids}}\right)$	3.02	1.30	0.50
Unsaturation index	180.38	140.41	92.60

TABLE IV

THE EFFECT OF THERMAL ACCLIMATION ON THE PERCENT DISTRIBUTION OF FATTY ACIDS IN TOTAL PHOSPHOLIPID OF BRAIN OF *C. VERSICOLOR*

Figures are presented as means ± S.E.

Fatty acids		Distribution (%) at acclimation temperature		
		16°C	26°C	36°C
Saturated				
14:0		0.14 ± 0.01 [a]	0.17 ± 0.005 [a]	0.21 ± 0.01 [a]
16:0		25.31 ± 0.68	31.44 ± 0.29	35.87 ± 0.98
18:0		5.41 ± 0.42	11.99 ± 0.29	14.94 ± 0.82
20.0		0.16 ± 0.003	3.31 ± 0.20	6.54 ± 0.32
Unsaturated				
Monounsaturated				
$n-9$	16:1	4.95 ± 0.32 [b]	4.45 ± 0.31 [b]	3.75 ± 0.31 [b]
	18:1	26.19 ± 0.83	21.72 ± 1.06	18.48 ± 0.72
	20:1	1.99 ± 0.55	1.96 ± 0.19	1.25 ± 0.16
Polyunsaturated				
$n-6$	18:2	10.46 ± 0.20	8.18 ± 0.96	6.52 ± 0.62
	20:2	0.80 ± 0.06 [c]	0.75 ± 0.06 [c]	0.70 ± 0.03 [c]
	20:3	0.77 ± 0.11 [d]	0.72 ± 0.02 [d]	0.68 ± 0.02 [d]
	20:4	9.82 ± 0.61	4.41 ± 0.55	1.18 ± 0.47
	22:4	0.71 ± 0.10 [e]	0.75 ± 0.01 [e]	0.76 ± 0.06 [e]
	22:5	0.71 ± 0.04	0.41 ± 0.03	0.28 ± 0.02
$n-3$	18:3	0.22 ± 0.02 [f]	0.16 ± 0.02 [f]	0.19 ± 0.005 [f]
	20:5	0.81 ± 0.05	0.60 ± 0.04	0.45 ± 0.03
	22:6	12.47 ± 1.20	8.91 ± 1.40	7.32 ± 0.46

[a–f], not significantly different at the 0.05 level of probablity (SNK test).

	Acclimation temperature		
	16°C	26°C	36°C
Ratio $\left(\frac{\%\text{ unsaturated fatty acids}}{\%\text{ saturated fatty acids}}\right)$	2.25	1.13	0.74
Unsaturation index	181.16	124.78	95.86

TABLE V

THE EFFECT OF THERMAL ACCLIMATION ON THE PHASE TRANSITION TEMPERATURE OF HYPOTHALAMIC MEMBRANE PHOSPHOLIPID OF *C. VERSICOLOR*

Acclimation temperature (°C)	Calorimetric data			
	Phase transition temperature range (°C)	Lower boundary of temperature range (t_s) (°C)	Upper boundary of temperature range (t_1) (°C)	Phase transition midpoint temperature (t_m) (°C)
16	11.0–19.0	11.0	19.0	16.0
26	23.0–30.0	23.0	30.0	26.0
36	38.0–47.0	38.0	47.0	42.0

TABLE VI

THE EFFECT OF THERMAL ACCLIMATION ON THE PHASE TRANSITION TEMPERATURES OF BRAIN MEMBRANE PHOSPHOLIPID OF *C. VERSICOLOR*

Acclimation temperature (°C)	Calorimetric data			
	Phase transition temperature range (°C)	Lower boundary of temperature range (t_s) (°C)	Upper boundary of temperature range (t_1) (°C)	Phase transition midpoint temperature range (t_m) °C)
16	0.58– 3.0	0.58	3.0	2.0
26	3.0 –12.0	3.0	12.0	9.0
36	27.0 –40.0	27.0	40.0	32.0

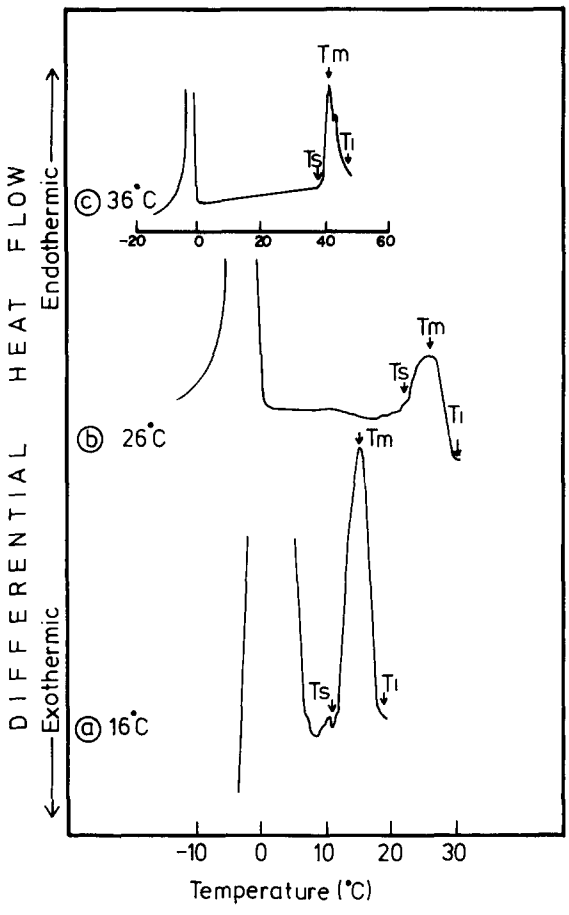

Fig. 1. Temperature-base thermograms of hypothalamic phospholipid membranes of *C. versicolor* acclimated to (a) 16°C, (b) 26°C and (c) 36°C). The lower boundary (t_s), transition midpoint (t_m) and upper boundary (t_1) of the phase transitions are denoted by the arrows.

melting of ice in the sample and the other transitions represent the crystalline to liquid-crystalline phase transition of the membrane phospholipids. When the acclimation temperature was 16°C, the phase-transition temperature range (t_s-t_1) of hypothalamic membrane phospholipid was from 11 to 19°C with a midpoint temperature (t_m) of 16°C. At 26°C acclimation temperature, the range was from 23 to 30°C with a t_m of 26°C. Again at 36°C acclimation temperature, the range was from 38 to 47°C with a t_m of 42°C.

But in the case of brain the corresponding values for 16°C and 26°C and 36°C acclimation temperatures were 0.58–3°C ($t_m - 2$°C), 3–12°C ($t_m - 9$°C) and 27–40°C ($t_m - 32$°C), respectively.

Discussion

A positive correlation (0.991) has been demonstrated for the first time between the phase-transition temperatures of the membrane phospholipids of hypothalamus and acclimation temperature, the t_m coinciding with acclimation temperature at 16°C and 26°C with a slight increase of t_m at 36°C. But no such relationship was noticed between acclimation temperature and the phospholipid phase-transition temperature of brain. This clearly confirmed the fact that hypothalamus is the most thermosensitive part of the brain, by providing a biophysical and molecular basis for the established neurophysiological concept.

Phase transition is associated with the melting of fatty acid hydrocarbon chains of the phospholipid. When phospholipid contains fatty acids

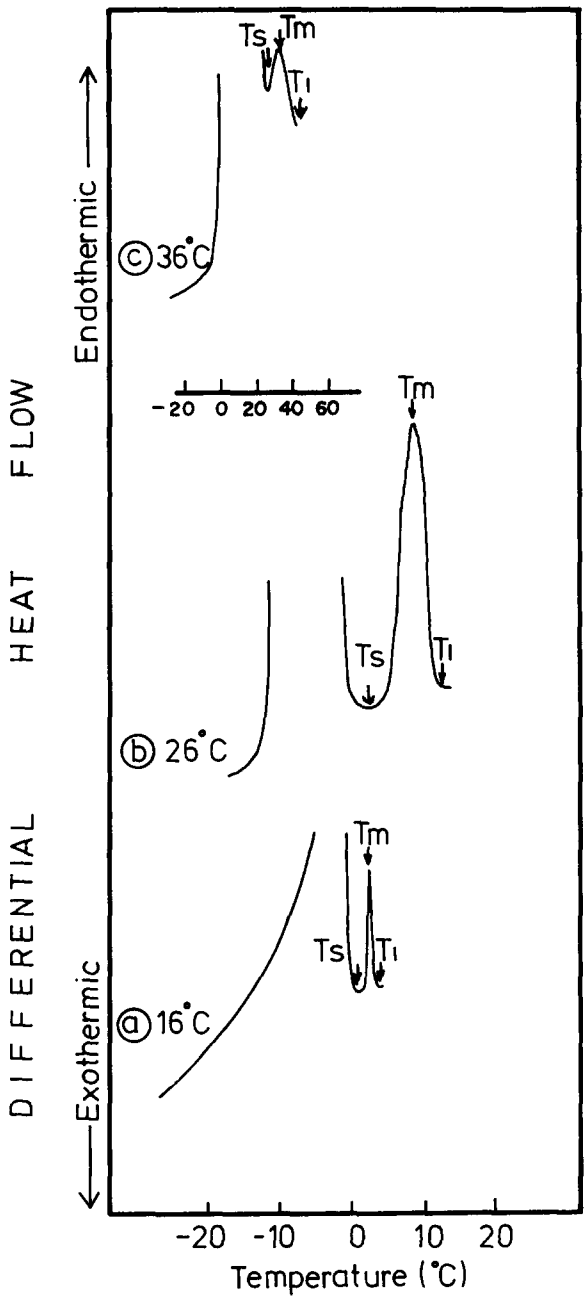

Fig. 2. Temperature base thermograms of brain phospholipid membranes of *C. versicolor* acclimated to (a) 16°C, (b) 26°C and (c) 36°C. The lower boundary (t_s), transition midpoint (t_m) and upper boundary (t_l) of the phase transitions are denoted by the arrows.

of shorter chain lengths or unsaturated bonds, the endothermic phase transition occurs at lower temperatures. The transition temperatures are high for the fully saturated long-chain fatty acid containing phospholipids [29]. This phenomenon is indicated by analysis of the phospholipid fatty-acid distribution in hypothalamus and brain of *C. versicolor* acclimated at 16, 26 and 36°C. The elevated phase-transition temperatures of hypothalamic membrane phospholipid at high acclimation temperature, were due to 2–3-fold increase of saturated fatty acids, as well as a decrease of low melting point unsaturated fatty acids of $n-9$, $n-6$ and $n-3$ series by 40–60% in the hypothalamus (and about 30–55% in the brain). The data provide a direct biochemical support for the thermoregulatory mechanisms as well as to the question of origin of thermoregulation. Mediation is by modulation of the fluidity of phospholipid membranes (saturation and unsaturation of fatty acids of phospholipid) in response to changing ambient temperature which regulates the 'on and off' mechanism of thermoregulatory control.

Increase in the percentage distribution of unsaturated fatty acids ($n-9=35\%$, $n-6=24\%$, $n-3=14\%$) during cold acclimation and decrease of saturated fatty acids altering the enoic to anoic acid ratio provide further evidence for phase transition temperature changes. Within the phase-transition temperature range, various proportions of gel and liquid-crystalline phases exist simultaneously so that the lipid bilayers are heterogeneous with respect to the physical state [30]. The narrow transition temperatures recorded in the present study could be due to the fact that head groups are less heterogeneous.

The high mortality of animals observed during hot acclimation in the present study could be seen along with the shift noticed in phase-transition temperatures ($t_m - 42$°C, while acclimation temperature was 36°C). Such an occurrence might have resulted in more liquid-crystalline membranes, which would be responsible for cell leakage, change in active transport and other membrane associated functions, eventually leading to cell death and thus that of the organism.

In *Nitella flexilis*, the temperature dependence of the action potential was related to the lipid phase transition of the membranes [31]. Speculations were made linking the effects of metal ions on lipid phase transitions with ideas about the mechanisms of nerve excitation, where abrupt co-

operative conformational changes occur in the axonal membrane during excitation. Cations directly affect the structure of presynaptic or axonal membranes to enhance or reduce nerve activity by affecting lipids [32]. Phase transitions of lipids determine the proper fluidity of cell membrane and perfect lipid phase separation characteristics, which in turn could regulate membrane properties like elasticity, insertion, aggregation, diffusional movements of the protein and lipid components as well as permeability characteristics leading to the physiological homeostasis during thermal acclimation in animals like *C. versicolor*.

Acknowledgements

This investigation was supported by Research grants to Professor G.Durairaj from the University Grants Commission, New Delhi, India. The authors are thankful to Professor C.N.R. Rao (I.I.Sc., Bangalore) for providing DSC facility.

References

1 Luzzati, V. and Husson, F.J. (1962) J. Cell Biol. 12, 217–219
2 Reiss-Husson, F. and Luzzati, V. (1967) Adv. Biol. Med. Phys. 11, 87–107
3 Melchior, D.L. and Steim, J.M. (1976) Annu. Rev. Biophys. Bioeng. 5, 205–238
4 Blazyk, J.E. and Steim, J.M. (1972) Biochim. Biophys. Acta 266, 737–741
5 Blume, A. (1979) Biochim. Biophys. Acta 557, 32–44
6 Demel, R.A., Jansen, J.W.C.M., Van Dijck, P.W.M. and Van Deenen, L.L.M. (1977) Biochim. Biophys. Acta 465, 1–10
7 Gawrisch, K., Arnold, K., Ruger, H.J., Kertscher, P. and Nuhn, P. (1977) Chem. Phys. Lipids 20, 285–293
8 Steim, J.M. (1968) in Molecular Association in Biological and Related Systems (Gould, R.F., ed.), pp. 259–302, Am. Chem. Soc. Washington, D.C.
9 Steim, J.M., Tourtellotte, M.E., Reinert, J.C., McElhaney, R.N. and Radar, R.L. (1969) Proc. Natl. Acad. Sci. U.S.A. 63, 104–109
10 Melchior, D.L., Morowitz, H.J., Sturtevant, J.M. and Tsong, T.Y. (1970) Biochim. Biophys. Acta 219, 114–122
11 De Kruyff, B., Demel, R.A. and Van Deenen, L.L.M. (1972) Biochim. Biophys. Acta 255, 331–347
12 Baldassare, J.J., Rhinehart, K.B. and Silbert, D.F. (1976) Biochemistry 15, 2986–2994
13 Ashe, G.B. and Steim, J.M. (1971) Biochim. Biophys. Acta 233, 810–814
14 Abbas, C.A. and Card, G.L. (1980) Biochim. Biophys. Acta 602, 469–477
15 Mason, W.T. and Abrahamson, E.W. (1974) J. Membrane Biol. 15, 383
16 Overath, P., Shairer, H.V. and Stoffel, W. (1970) Proc. Natl. Acad. Sci. U.S.A. 67, 606–612
17 Tsukagoshi, N. and Fox, C.F. (1973) Biochemistry 12, 2820–2822
18 Kimelberg, H.K. and Papahadjopoulos, D. (1972) Biochim. Biophys. Acta 282, 277–292
19 Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497–509
20 Rouser, G., Kritchevski, G., Simon, G. and Nelson, G.J. (1967) Lipids 2, 37–40
21 Fiske, C.H. and SubbaRow, Y. (1925) J. Biol. Chem. 66, 375–400
22 Rouser, G., Siakotos, A.N. and Fleischer, S. (1966) Lipids 1, 85–86
23 Morrison, W.R. and Smith, L.M. (1964) J. Lipid Res. 5, 600–608
24 Walker, B.L. (1969) J. Nutr. 94, 469–474
25 Durairaj, G. and Martin, E.W. (1975) Comp. Biochem. Physiol. 50B, 237–248
26 Brandt, A.E. and Lands, W.E.M. (1968) Lipids 3, 178–181
27 Carroll, K.K. (1961) Nature 191, 377–378
28 Sokal, R.R. and Rohlf, F.J. (1969) Biometry. The Principles and Practice of Statistics in Biological Research, W.H. Freeman, San Francisco
29 Byrne, P. and Chapman, D. (1964) Nature 202, 987–988
30 Oldfield, E. and Chapman, D. (1972) FEBS Lett. 23, 285–297
31 Blatt, F.J. (1974) Biochim. Biophys. Acta 339, 382–389
32 Trauble, H. and Eibl, H. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 214–219