Biochimica et Biophysica Acta, 687 (1982) 303–309
Elsevier Biomedical Press

BBA 71165

VARIABLE HOMEOVISCOUS RESPONSES OF DIFFERENT BRAIN MEMBRANES OF THERMALLY-ACCLIMATED GOLDFISH

ANDREW R. COSSINS * and C. LADD PROSSER

Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801 (U.S.A.)

(Received October 29th, 1981)

Key words: Homeoviscous response; Thermal adaptation; Membrane fluidity; (Goldfish brain)

The effects of thermal acclimation of goldfish upon the bulk fluidity of synaptic, mitochondrial and myelin membrane fractions of brain was determined using steady-state and differential polarised phase fluorimetry. Membrane fluidity decreased in the order, mitochondria>synaptic membranes>myelin. In each case membranes from cold-acclimated goldfish were more fluid than the corresponding membranes of warm-acclimated goldfish, though the adjustment of fluidity in each case was insufficient to compensate for the direct effects of the temperature difference. The extent of fluidity compensation was greatest in the mitochondrial fraction and least in the myelin fraction, indicating heterogeneous responses in different membrane-types. Steady-state and dynamic fluorimetric techniques provided identical estimates of the homeoviscous responses, indicating that despite its short-comings, the steady-state technique provided as good a measure of adaptive responses as the more complex and sophisticated technique.

Introduction

The adjustment of membrane fluidity to offset or compensate for the direct effects of cellular temperature changes appears to be a widespread cellular mechanism of temperature adaptation [1,2]. The adaptive value of this response is thought to be related to the preservation of membrane dynamic structure in the face of environmental variability, and hence to the constancy of processes and functions of cellular membranes that are influenced by membrane fluidity. An ideal or perfect response [3] would occur when membrane fluidity had identical adapted values at different adaptation temperatures. In principle, this could be achieved either by a rotation of the fluidity versus temperature curves during adaptation to an altered temperature and/or by the movement of the curve along the temperature axis (a so-called translation, Ref. 3). Of the homeoviscous responses reported for the temperature adaptation of eukaryotes, all have been non-ideal translations [4–7].

It may well be that homeoviscous responses in eukaryotes are characteristically non-ideal, and that ideal responses are either not possible or have no adaptive value. However, the sheer complexity of membrane systems suggests that there may be other explanations. For example, current techniques for quantifying the degree of membrane fluidity provide a weighted average value for all hydrophobic sites sampled by the population of spectroscopic probes. Thus, an impure membrane preparation may exhibit a non-ideal response whilst each component membrane-type may have a considerably different response, some of which may be ideal and others non-ideal. Furthermore, the microheterogeneity of membranes that may be caused perhaps by the so-called 'boundary' layer,

* Present address: Department of Zoology, University of Liverpool, Liverpool, L69 3BX, U.K.

Abbreviation: DPH, 1,6-diphenylhexatriene.

by phase separations [8] or by microcrystalline 'clusters' [9] may result in each region of the membrane having a distinct responsiveness during thermal adaptation.

Whilst it is not possible, at present, to describe this microheterogeneity in particular membranes to any great extent, it is possible to subfractionate membrane preparations and to determine the responsiveness of each subfraction. We have accordingly subfractionated the brain synaptosomes of thermally-acclimated goldfish, the subject of an earlier study [5]. The responsiveness of each subfraction was determined by measuring the difference in membrane fluidity using steady-state and differential polarised phase fluorimetry. Our results demonstrate that myelin was modified only slightly as a result of thermal treatment, whilst synaptic membranes and mitochondria showed a considerably greater response.

Methods

Animals. Goldfish (*Carassius auratus*, 5–7 inch) were obtained from a commercial source and acclimated to 7°C or 28°C for at least 21 days as described by Cossins [5].

Fractionation of brain membranes. All procedures were carried out at 0–4°C. Goldfish were killed by bisection of the spinal cord and their brains were rapidly dissected out and freed of fatty material, blood clots and spinal cord. The brains of 5–10 fish were homogenised in 10 ml ice-cold isolation medium (280 mM sucrose, 2 mM EDTA, 20 mM imidazole pH 7.4 at room temperature) using 10–15 passes of a glass-Teflon homogeniser (Thomas type C). The homogenate was spun at $1000 \times g$ for 10 min and the pellet was resuspended in 5 ml isolation medium, homogenised and then centrifuged again at $1000 \times g$ for 10 min. The supernatants from both low-speed centrifugations were pooled and centrifuged at $12000 \times g$ for 30 min. The upper white portion of the pellet was gently resuspended in 15 ml lysing medium (1 mM EDTA, 10 mM imidazole-HCl, pH 7.4 at room temperature) leaving a hard brown pellet. The suspension was then homogenised in a tight-fitting all-glass Dounce homogeniser (Kontes, 'A' pestle) and left to stand for 30 min. The lysed suspension was centrifuged at $18000 \times g$ for 30 min. The pellets were resuspended in isolation medium and layered on discontinuous sucrose gradient consisting of 1.2 M, 1.0 M, 0.9 M and 0.8 M sucrose in 10 mM imidazole, pH 7.4 at room temperature. The gradients were centrifuged at 27000 rev./min in a Beckman SW 27 rotor. The myelin fraction (interface of isolation medium and 0.8 M sucrose) and the synaptic membrane fraction (interface of 0.9/1.0 and 1.0/1.2 M sucrose combined) were removed with Pasteur pipettes and diluted to approx. 300 mosM with lysing medium. The hard, brown mitochondrial pellet was resuspended in isolation medium and gently homogenised with a small hand-held Dounce homogeniser. Each fraction was then centrifuged at $18000 \times g$ for 45 min and the resulting pellets were resuspended in a small volume of lysing medium.

Labelling of membrane fractions. Myelin, synaptic and mitochondrial membrane fractions were labelled with DPH as described previously [5].

Fluorimetry. Polarisation and fluorescence lifetimes were measured as described previously [5,7]. Differential lifetimes were measured at a modulation frequency of 18 MHz exactly as described by Cossins et al. [7]. In all experiments scattered light was approx. 0.5% of total emitted light or less and corrections were not necessary. Some 'colour effect' [10] was noted with the Philips XP2230 photomultipliers used in the phase/modulation fluorometer, due to the difference in kinetic energy of the photoelectrons excited by scattered light (360 mm) and fluorescent light (> 400 mm). This artefact was corrected in all subsequent calculations using the method described by Jameson and Weber [11] with phase and modulation measurements obtained at modulation frequencies 6 MHz and 18 MHz.

Results

The effects of temperature upon the polarisation and differential lifetime ($\Delta\tau$) of DPH in brain membranes of thermally acclimated goldfish are presented in Figs. 1 and 2, respectively. Mitochondria displayed the lowest values of polarisation and myelin the highest. In all fractions, the values of polarisation of membranes of 7°C-acclimated goldfish were lower than for the

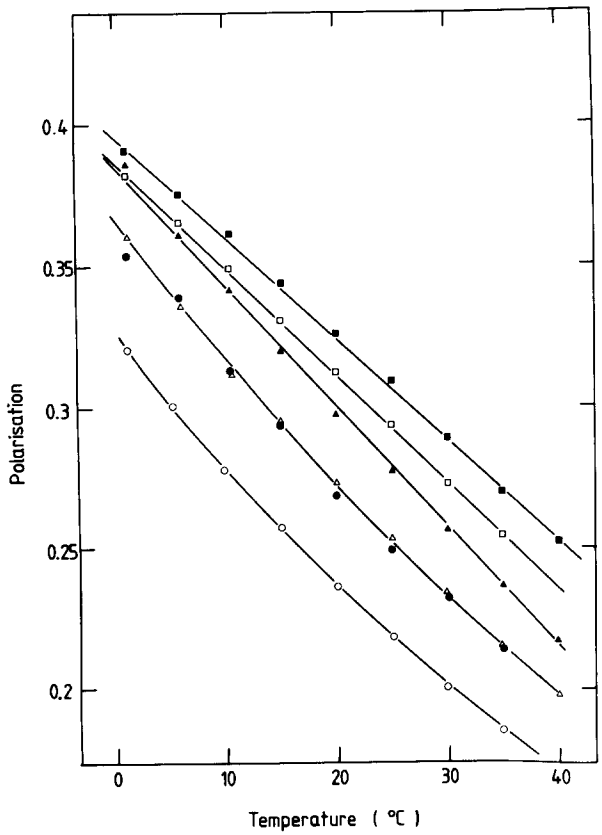

Fig. 1. The effect of temperature upon the fluorescence polarisation for brain membrane fractions of thermally-acclimated goldfish. ▲, △, synaptic membranes; ●, ○, mitochondria; ■, □, myelin; closed symbols, −28°C-acclimated goldfish; open symbols, 7°C-acclimated goldfish.

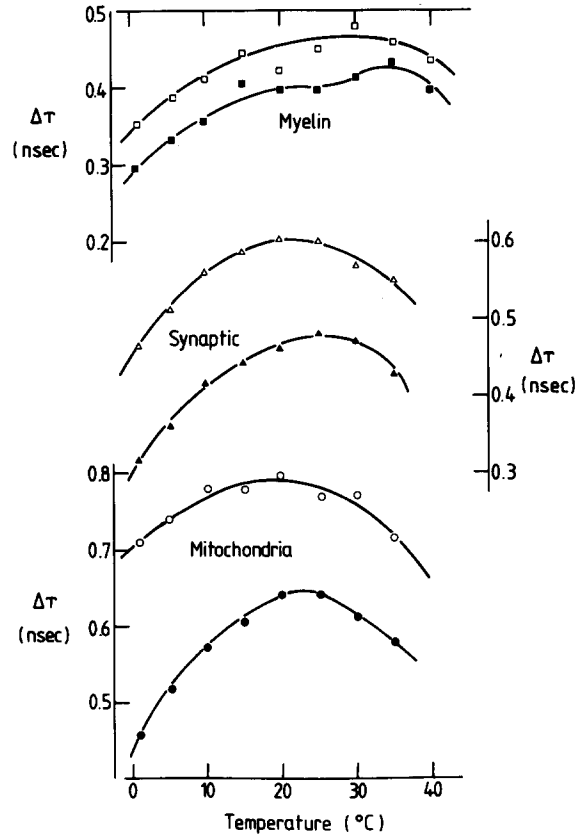

Fig. 2. The effect of temperature upon the differential lifetime ($\Delta\tau$) for brain membrane fractions of thermally-acclimated goldfish. The scale for the ordinate has been staggered to separate the curves for each membrane fraction. Symbols as in Fig. 1.

corresponding membranes of 28°C acclimated goldfish. The largest differences were observed in the mitochondrial fraction and the smallest differences in the myelin fraction.

The temperature dependence of $\Delta\tau$ for synaptic and mitochondrial membranes (Fig. 2) were similar to the flattened, bell-shaped curves observed previously in studies of model [12] and in natural membranes [7]. The curves for myelin were rather atypical in that there was no marked reduction in $\Delta\tau$ at high temperatures. The $\Delta\tau$ measured in the mitochondrial and synaptic membranes of 7°C-acclimated goldfish were substantially greater than for the corresponding preparations of 28°C-acclimated goldfish. The curves for myelin, however, were only slightly different. The maximal observed values of $\Delta\tau$ are presented in Table I, together with the corresponding theoretical values calculated assuming isotropic, unhindered rotations of the probe. In all fractions the observed $\Delta\tau$ achieved less than half of the theoretical value suggesting that probe rotation was highly constrained [7,12,13]. The disparity between observed and calculated $\Delta\tau$ was greatest in membrane preparations of 28°C-acclimated goldfish, indicating a greater constraint of probe motion in these membranes and by inference, a greater degree of order.

The data derived from steady state measurements were analysed, according to the Perrin equation to yield a rotational correlation coefficient, $\bar{R}$ (Fig. 3). $\bar{R}$ was greatest in mitochondrial membranes and smallest in myelin membranes. Values

TABLE I

TANGENT DEFECTS FOR THE VARIOUS MEMBRANE PREPARATIONS OF BRAINS ISOLATED FROM 7°C- AND 28°C-ACCLIMATED GOLDFISH

Membrane fraction	$\Delta\tau_{max}$ (ns)		Tangent defect (%)
	Obs.	Calcd.	
7°C Synaptic membrane	0.60 [a]	2.11 [b]	28 [c]
28°C Synaptic membrane	0.48	2.16	22
7°C Mitochondria	0.79	1.94	41
28°C Mitochondria	0.65	2.01	32
7°C Myelin	0.46	2.20	21
28°C Myelin	0.43	2.25	19

[a] Observed $\Delta\tau_{max}$ at 25°C obtained from Fig. 2. Each value was obtained from experiments on a single preparation though they were typical of results from two other more incomplete studies.

[b] Calculated $\Delta\tau_{max}$ calculated according to Equation 11 of Lakowicz et al. (1979) [12].

[c] Calculated as (observed $\Delta\tau_{max}$/calculated $\Delta\tau_{max}$)×100.

for membranes of 7°C-acclimated goldfish were greater than for membranes of 28°C-acclimated goldfish. In addition, the data from both steady-state and differential polarised phase measurements were combined and analysed using a 'wobbling-in-cone' model, as described by Weber [13] and modified by Lakowicz et al. [12], to yield the limiting anisotropy r_∞, and the rotational rate, R. The limiting anisotropy, r_∞, is the lowest value of anisotropy attainable at times that are long compared to the fluorescent lifetime and it reflects the degree of constraint imposed upon the wobbling motion of the fluorophore by the anisotropic hydrocarbon environment. The rotational rate, R, reflects the average rate of motion of the probe within a cone that is defined by the hydrocarbon environment [13,14]. The effect of temperature upon r_∞ is illustrated in Fig. 4. The membranes from 7°C-acclimated goldfish displayed somewhat lower values of r_∞ than the corresponding mem-

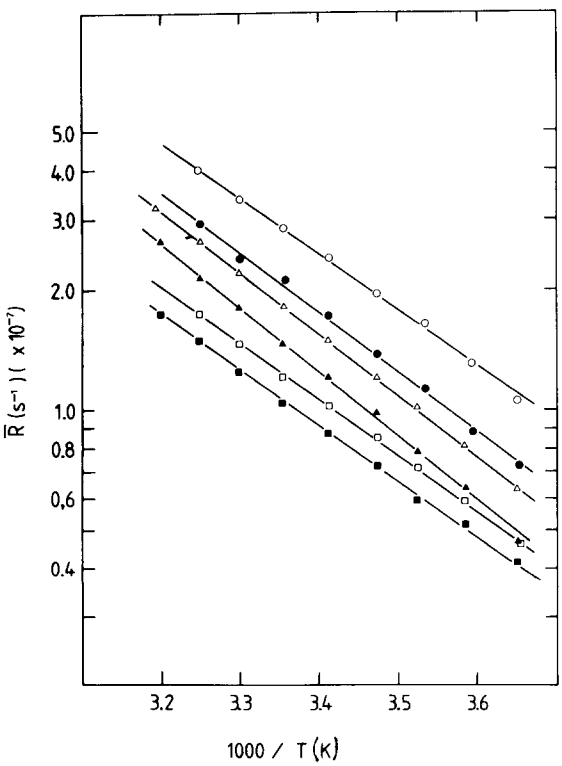

Fig. 3. Arrhenius plot of the rotational correlation coefficient ($\bar{R}$) for brain membrane fractions of thermally-acclimated goldfish. Symbols as in Fig. 1.

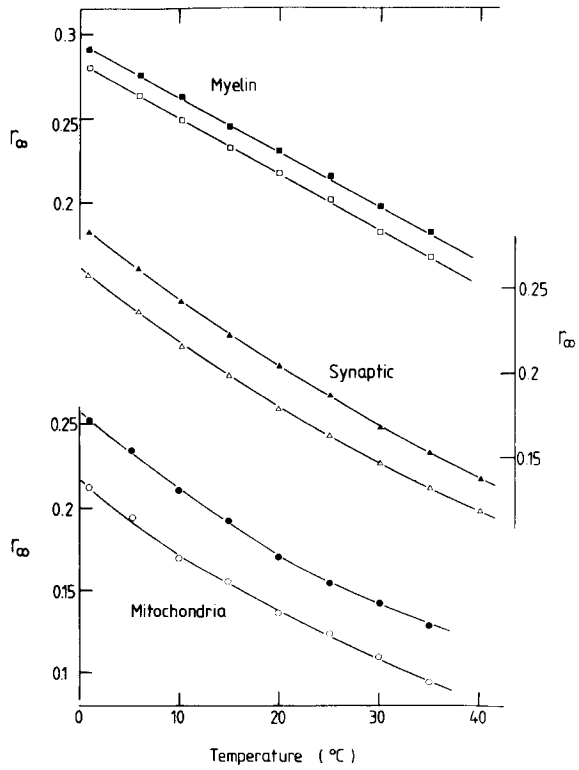

Fig. 4. The effect of temperature upon the limiting anisotropy (r_∞) for brain membranes of thermally-acclimated goldfish. Symbols as in Fig. 1.

TABLE II

A COMPARISON OF THE MEASURED AND DERIVED MOTIONAL PARAMETERS FOR DPH AT 5°C IN MEMBRANES ISOLATED FROM THE BRAINS OF 7°C AND 28°C ACCLIMATED GOLDFISH

Probability calculated for 't' test (two-tailed) for comparison of values for 7°C- and 28°C-acclimated goldfish.

	Polarisation	$\bar{R}$ (s^{-1}) ($\times 10^{-7}$)	R (s^{-1}) ($\times 10^{-8}$)	r_∞	$\Delta\tau$ (s) ($\times 10^{9}$)	τ (s) ($\times 10^{9}$)
Synaptic membranes						
7°C-acclimated ($n=5$)	0.333 [a]	0.840 [a]	1.190 [b]	0.232 [a]	0.509 [a]	10.77 [b]
	±0.001	±0.011	±0.002	±0.001	±0.009	±0.02
28°C-acclimated ($n=5$)	0.365	0.602	1.34	0.264	0.364	10.94
	±0.001	±0.009	±0.003	±0.002	±0.005	±0.04
Mitochondria						
7°C-acclimated ($n=4$)	0.302 [a]	1.320 [a]	0.964	0.193 [a]	0.744 [b]	9.16 [c]
	±0.002	±0.003	±0.023	±0.002	±0.020	±0.10
28°C-acclimated ($n=5$)	0.339	0.890	1.160	0.234	0.517	9.69
	±0.003	±0.040	±0.009	±0.004	±0.041	±0.14
Myelin						
7°C-acclimated ($n=5$)	0.360 [b]	0.632 [b]	1.204 [c]	0.258 [b]	0.420 [a]	10.98
	±0.003	±0.020	±0.002	±0.003	±0.010	±0.03
28°C-acclimated ($n=4$)	0.374	0.532	1.290	0.273	0.350	11.35
	±0.001	±0.008	±0.003	±0.001	±0.007	±0.20

[a] $P<0.001$.
[b] $P<0.01$.
[c] $P<0.05$.

branes of 28°C-acclimated goldfish.

The results of replicate measurements at 5°C are presented in Table II to demonstrate the reproducibility of differences between acclimation groups. In general, the values of polarisation, $\bar{R}$ and r_∞ that were obtained for 7°C- and 28°C-acclimated goldfish were significantly different. The differences in R were less significant in synaptic membranes and myelin or not significant in mitochondria.

A comparison of the magnitude of the fluidity differences of membranes from 7°C- and 28°C-acclimated goldfish using different estimates of fluidity is presented in Table III. As before (see Ref. 2), the responsiveness of the different membrane fractions has been calculated by measuring the shift in the position of the fluidity parameter versus temperature curve along the temperature axis as a result of acclimation and expressing it as a fraction of that shift required for an 'ideal' or 'complete' compensation of fluidity (hereafter termed the 'homeoviscous efficacy'). Thus an ideal response would yield an efficacy of 1.0 whilst a very small shift would yield an efficacy close to zero. It is interesting to note that the values obtained in the present study were virtually independent of the method used to estimate fluidity; thus

TABLE III

THE MAGNITUDE OF THE DIFFERENCES IN THE FLUIDITY OF BRAIN MEMBRANE FRACTIONS ISOLATED FROM 7°C- AND 28°C-ACCLIMATED GOLDFISH CALCULATED USING VARIOUS FLUIDITY PARAMETERS

Polarisation values were calculated as the fraction of the shift of the fluidity versus temperature curve (or $1/T$ in the case of $\bar{R}$) along the abscissa as a result of thermal acclimation, to that required for an ideal or complete compensation of fluidity.

Membrane fraction	Fluidity parameter		
	Polarisation	$\bar{R}$	r_∞
Synaptic membranes	0.35	0.36	0.37
Mitochondria	0.43	0.43	0.45
Myelin	0.19	0.22	0.22

polarisation yields the same information regarding the magnitude of the fluidity adjustment as do the more sophisticated fluidity parameters $\bar{R}$ and r_∞. The efficacy of each membrane fraction was distinctly different with mitochondria showing the greatest efficacy and myelin the least.

Discussion

All fluorescence measurements indicated that membrane fluidity was highest in the mitochondrial fraction and lowest in the myelin fraction. The steady state polarisation and tangent defect (Table III) was greatest in the myelin fraction compared to the other fractions, which is consistent with myelin possessing a particularly ordered bilayer. This difference in fluidity of brain fractions conforms to what one might expect on the basis of the known lipid composition and structural characteristics of brain membranes. Thus, myelin is particularly rich in cholesterol [15,16], whilst mitochondria generally have a very low cholesterol content.

The variable homeoviscous efficacy of various brain membrane fractions highlights the problems of estimating the true responsiveness of particular membrane-types. Virtually all membrane preparations are impure to a greater or lesser extent. Whilst the fractions used in the present study are quite distinct and are considerably less heterogeneous than those used in a previous study of brain membranes [5], it is still not possible to be confident that the adaptive properties of a single class of membranes have been unequivocally defined.

Nevertheless, the great disparity in the fluidity adjustments of different membrane fractions during thermal acclimation is consistent with earlier observations on liver membranes of green sunfish [7], and is consistent with the variable differences in lipid composition of brain membrane fractions of differently acclimated goldfish brain [17]. However, it should be pointed out that these measurements were made approximately 21–30 days after the acclimation treatment was started. Whilst studies of the time-course of fluidity adjustments in goldfish have confirmed that 21 days was sufficient to achieve a steady-state fluidity in brain synaptosomes [18] and probably in mitochondria, the same cannot be said of myelin. The rate of turnover of myelin components is likely to be substantially slower than in the other fractions and it may require a considerable period of time for myelin to exhibit a constant fluidity. Whether or not the variable efficacy of different membranes is due to differences in turnover times, the more rapid and extensive fluidity adjustments of mitochondrial and synaptic membranes may have some adaptive significance, since it is reasonable to expect that the particular functional properties of these membranes would be more responsive to perturbations of fluidity than would be the case in myelin.

Despite these uncertainties, it is clear that homeoviscous processes in fish are not, in general, capable of maintaining membrane fluidity constant at different acclimation temperatures, and the same is true of the protozoan, *Tetrahymena* [4]. However, this does not necessarily mean that homeoviscous responses are unimportant or of only limited adaptive value. Fluidity adjustment is probably only one of several adaptive responses which may influence membrane-associated processes. For example, the concentration of membrane-associated proteins may be modulated independently, as appears to be the case in cytochrome c and cytochrome oxidase of fish muscle [19]. This, together with the higher turnover number which presumably would result from a more fluid hydrophobic environment [20] may permit comparable catalytic rates at different acclimation temperatures. Alternatively, the arrangement of membrane systems with respect to cellular organelles may be altered to modify cellular diffusion distances for metabolites, activators or signals, as appears to be the case with the sarcoplasmic reticulum of fish epaxial muscle [21]. Finally, small fluidity compensations may be quite sufficient to overcome thermotropic transitions in membrane structure, which though not as profound as the gel to liquid-crystalline phase transitions observed in artificial membranes may have deleterious effects upon membrane function.

There is good reason, therefore, to expect that fluidity compensations that are complete or ideal, in the quantitative sense may not be the most appropriate adaptive response in every case.

Acknowledgments

Research support from NSF Grant PCM 74-15861 is acknowledged. A.R.C. was the recipient of a Wellcome Trust travel grant. We are grateful to Drs. G. Weber and D.M. Jameson for the use of fluorescence instrumentation.

References

1 Hazel, J. and Prosser, C.L. (1974) Physiol. Rev. 54, 620–677
2 Cossins, A.R. (1981) in Effects of Low Temperature on Biological Membranes (Clarke, A. and Morris, J., eds.), pp. 83–106, Academic Press, London
3 Precht, H., Christophersen, J., Hensel, H.and Larcher, W. (1973) Temperature and Life, Springer Verlag, Berlin
4 Nozawa, Y., Iida, H., Fukushima, H. and Ohnishi, S. (1974) Biochim. Biophys. Acta 367, 134–147
5 Cossins, A.R. (1977) Biochim. Biophys. Acta 470, 395–411
6 Cossins, A.R., Christiansen, J.A. and Prosser, C.L. (1978) Biochim. Biophys. Acta 511, 442–454
7 Cossins, A.R., Kent, J. and Prosser, C.L. (1980) Biochim. Biophys. Acta 599, 341–358
8 Kleeman, W., Grant, C.W.M. and McConnell, H.M. (1974) J. Supramol. Struct. 2, 609–616
9 Lee, A.G. (1975) Prog.Biophys. Mol. Biol. 29, 3–56
10 Rayner, D.M., McKinnon, A.E., Szabo, A.G. and Hackett, P.A. (1976) Can. J. Chem. 54, 3246
11 Jameson, D. and Weber, G. (1981) J. Chem. Phys. 85, 953–958
12 Lakowicz, J.R., Prendergast, F.G. and Hogan, D. (1979) Biochemistry 18, 508–519
13 Weber, G. (1978) Acta Physiol. Pol. A54, 173
14 Kinosita, A. Jr., Kawato, S. and Ikegami, A. (1977) Biophys. J. 20, 289–305
15 Norton, W.T. and Autilio, L.A. (1966) J. Neurochem. 13, 213–222
16 Selivonchick, D.P. and Roots, B.I. (1976) J. Therm. Biol. 1, 131–135
17 Selivonchick, D.P., Johnston, P.V. and Roots, B.I. (1977) Neurochem. Res. 2, 379–393
18 Cossins, A.R., Friedlander, M.J. and Prosser, C.L. (1977) J. Comp. Physiol. 120, 109–121
19 Sidell, B. (1977) J. Exp. Zool. 199, 233–250
20 Sinensky, M., Pinkerton, F., Sutherland, E. and Simon, F.R. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 4893–4897
21 Penney, R.K. and Goldspink, G. (1980) J. Therm. Biol. 5, 63–68