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ADAPTATION OF BIOLOGICAL MEMBRANES TO TEMPERATURE

THE LACK OF HOMEOVISCOUS ADAPTATION IN THE SARCOPLASMIC RETICULUM

ANDREW R. COSSINS *, JAMES CHRISTIANSEN and C. LADD PROSSER

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

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Summary

Temperature adaptation of biological membranes was examined by comparing the fragmented sarcoplasmic reticulum preparation of goldfish acclimated to different temperatures. Membrane fluidity was estimated using the fluorescence polarization technique. There was considerable variation between preparations, but no consistent differences in fluidity were observed between 5- and 25°C-acclimated goldfish, fish species adapted over an evolutionary period to arctic or desert temperatures, and rat. The fatty acid composition of the sarcoplasmic reticulum preparations of differently acclimated goldfish showed differences in the proportion of mono- and polyunsaturated fatty acids while the proportion of saturated fatty acids remained relatively constant. However, the fatty acid composition of sarcoplasmic reticulum phosphoglycerides became more unsaturated in the order rat, desert pupfish, arctic sculpin, which correlates with their respective environmental or body temperature. It is concluded that differences in membrane components other than fatty acids are important in determining membrane dynamic structure. The inability to demonstrate homeoviscous adaptation in sarcoplasmic reticulum is supported by other evidence suggesting that functions of the sarcoplasmic reticulum that are measured in vitro are not affected by such modifications of their phosphoglyceride fatty acid composition as occur during thermal acclimation.

Introduction

A general response of organisms to a decreased environmental temperature is the incorporation of greater proportions of *cis*-unsaturated hydrocarbons

* To whom correspondence should be addressed at Department of Zoology, University of Liverpool, Liverpool L69 3BX, U.K.

Abbreviation: DPH, 1,6-diphenyl-1,3,5-hexatriene.

into membrane phospholipids [1,2]. Largely as a result of studies with model membrane systems composed of defined phospholipids [3], this shift in fatty acid composition during cold acclimation has been interpreted as a homeostatic response which compensates membrane structure and function for the direct effects of the temperature shift, and has been termed "homeoviscous adaptation" [4—6]. This hypothesis has been supported by indirect examination of the dynamic nature of membranes isolated from organisms which have become acclimated to different environmental temperatures. Thus, membranes of *Escherichia coli* [4], *Bacillus stearothermophilus* [7], *Tetrahymena pyriformis* [8], and brain synaptosomes of goldfish [6] all exhibited increased membrane fluidity at a given measurement temperature after acclimation to lower environmental temperatures and vice versa. Cossins and Prosser [9] have recently extrapolated the relationship between cell temperature, lipid composition and membrane viscosity to include other fish species adapted to diverse thermal environments as well as small mammals.

The extent of homeoviscous adaptation varied according to the organism. In this respect, bacteria exhibited a perfect adaptation since membrane fluidity was almost identical at each of the different growth temperatures [4,7]. In *Tetrahymena* [8] and in goldfish brain synaptosomes, however, the adaptation was less perfect and this correlated with the less extensive alteration of membrane lipid composition in these organisms than in prokaryotes. It has been suggested [6] that the partial response in goldfish brain synaptosomes was due to the presence of several distinct membrane types which show different degrees of compensation, the net result being a weighted average for the membrane preparation. This suggests that some membrane types were not affected during acclimation to different temperatures. A membrane preparation composed of only one membrane type, such as the fragmented sarcoplasmic reticulum preparation of skeletal muscle, provides a more suitable subject for analysis. This membranous preparation is derived from a complex system of inter-connected vesicles which act as an intracellular store of Ca^{2+}, releasing it during muscle contraction and sequestering it during muscle relaxation through the action of a membrane-bound calcium pump, the Ca^{2+}-stimulated ATPase. We present here an analysis of the effects of thermal acclimation of goldfish on the viscosity and fatty acid composition of their sarcoplasmic reticulum membranes, and comparative experiments with rat and with fish species adapted to diverse thermal environments.

Methods

Animals. Goldfish (*Carassius auratus*, 12—18 cm) were obtained from a commercial source and were maintained at 5, 15 or 25°C for at least 21 days, as described previously [6]. Arctic Sculpin (*Myoxocephalus* sp., 30 cm) were caught in the Bering Sea at 1°C and were maintained in the laboratory at 0.5 ± 1°C in artificial sea water for several days. Desert Pupfish (*Cyprinodon nevadensis*, 3—4 cm) were reared at approximately 28°C from stocks originally obtained from Death Valley, California. On arrival in Illinois they were kept at 28°C for 2 days and slowly warmed over a four day period to 34 ± 0.5°C where they were kept for seven days before being killed. Pupfish were fed 5—6

times daily with 'TetraMin' fish flakes (Tetra Werke Gmbh, West Germany). Rats (Wistar Strain, 250—300 g) were laboratory reared and fed ad libitum with Purina Rat Chow.

Isolation of sarcoplasmic reticulum preparations. All procedures were performed at 0—4° C. Fish were killed by decapitation and the white epaxial muscle was rapidly excised, taking care to remove all traces of red muscle. Each muscle block was finely chopped, minced with 10 vols. (w/v) ice-cold extraction medium (100 mM KCl/10 mM imidazole-HCl, pH 7.1) in a Waring Blendor operating at maximum speed for 15 s and finally homogenized with 10 passes of a glass-Teflon homogenizer. The crude muscle homogenate was centrifuged at 1200 × *g* for 10 min in a Beckman JA-20 rotor and the supernatant was decanted and centrifuged at 15 000 × *g* for 30 min to remove mitochondria and myofibrillar material. The supernatant was centrifuged for a further 60 min at 48 000 × *g* and the pellet was re-suspended in 0.6 M KCl/10 mM imidazole-HCl, pH 7.1, by 8 passes in a glass-Teflon homogenizer to solubilize contaminating actomyosin filaments [10]. The sarcoplasmic reticulum preparation was pelleted by centrifugation at 48 000 × *g* for 60 min, re-suspended in a small volume of isolation medium by gentle hand homogenization in an all-glass apparatus and used immediately. Rats were killed with a blow to the head and the gastrocnemius muscle was treated similarly.

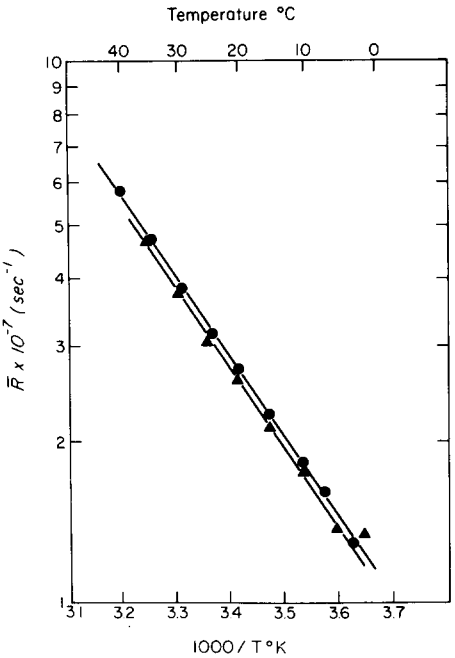
Lipid analyses. Phospholipids were extracted using chloroform/methanol (2 : 1, w/v) with 0.005% 2,6-di-*tert*-butyl-*p*-cresol, purified by two-dimensional thin layer chromatography and their constituent fatty acids analysed by gas liquid chromatography, as described previously [6].

Fluorescence polarization analyses. Fluorescence polarization measurements were made on a T-format photon-counting fluorescence polarization photometer [11] and the rotational diffusion coefficient ($\overline{R}$), and derived microviscosity (η) were calculated as described previously [6]. Fluorescence lifetimes (τ) were measured using a modified version of the cross correlation phase fluorometer described by Spencer and Weber [12] at a modulation frequency of 10 MHz.

Results

Goldfish acclimated at 5 and 25° C. Fig. 1 presents typical Arrhenius plots of $\overline{R}$ for 1,6-diphenyl-1,3,5-hexatriene (DPH) incorporated into sarcoplasmic reticulum preparations isolated from 5 and 25° C-acclimated goldfish. At higher measurement temperatures, $\overline{R}$ increased, indicating a less restrictive probe environment. In all experiments the Arrhenius plots yielded straight lines with correlation coefficients in excess of 0.98. The average results for experiments on a number of membrane preparations of both acclimation groups are compared in Table I, together with values for microviscosity for comparative purposes.

There was considerable variation in $\overline{R}$ for different preparations within each acclimation group and overlap in the range of values for each acclimation group precluded any consistent or significant difference between them. Despite the variation in $\overline{R}$, its temperature dependence proved quite consistent, with activation energies of approximately 6 kcal · mol^{-1} (Table I). The differences



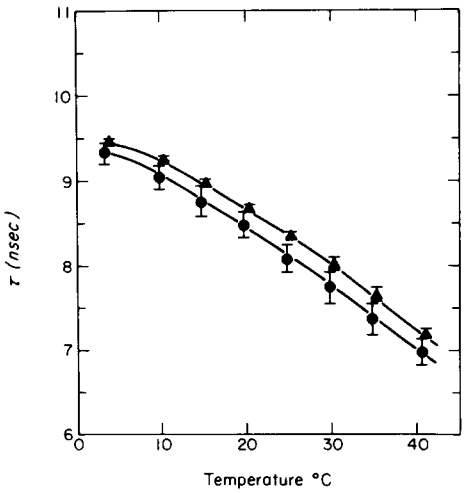


Fig. 1. Typical Arrhenius plots of $\bar{R}$ for DPH in sarcoplasmic reticulum membranes isolated from 5°C (●) and 25°C- (▲) acclimated goldfish. $\bar{R}$ was calculated from polarization and lifetime data as described in Methods.

Fig. 2. Temperature dependence of mean fluorescence lifetime for DPH in sarcoplasmic reticulum membranes isolated from 5°C- (●) and 25°C- (▲) acclimated goldfish. Lifetimes derived by the phase and modulation methods for each preparation were averaged. The values represent the mean fluorescence lifetime for three preparations for each acclimation group and bars denote the standard error of the mean.

in activation energy between 5- and 25°C-acclimated goldfish were not statistically significant.

The fluorescent lifetime of DPH in sarcoplasmic reticulum preparations of 5- and 25°C-acclimated goldfish was measured in a separate series of experiments. The average lifetime values measured by the modulation technique were consistently greater by approximately 0.6 ns than those measured by phase techniques. This is interpreted as a small degree of heterogeneity of probe environments (ref. 6 and Weber, G., unpublished). The difference in average values for the 5- and 25°C-acclimated goldfish were not statistically significant (Table II). With decreased assay temperature the fluorescence lifetime increased (Table II; Fig. 2) and by extrapolation reached a plateau of approximatetly 0°C for preparations of both 5- and 25°C-acclimated goldfish.

The fatty acid compositions of the choline, ethanolamine and serine/inositol phosphoglycerides for 5- and 25°C-acclimated goldfish are presented in Table III. In all three fractions there were conspicuous differences between the acclimation groups. In the choline phosphoglycerides, for example, the proportion of 18 : 1 in 25°C-acclimated goldfish was almost twice that of 5°C-acclimated goldfish, while 18 : 2 ω 6 showed the reverse. The fatty acid compositions are summarized in Table III as the total saturated, monounsaturated or polyunsatu-

TABLE I

AVERAGE ROTATIONAL DIFFUSION COEFFICIENT ($\bar{R}$) AND MICROVISCOSITY (η) AT 5, 20 AND 35°C AND ACTIVATION ENERGY (E_a) FOR DPH IN SARCOPLASMIC RETICULUM PREPARATIONS ISOLATED FROM THE SKELETAL MUSCLE OF 5 AND 25°C-ACCLIMATED GOLDFISH

Values represent the mean ± S.E.; n, number of preparations.

	$\bar{R} \times 10^{-7}$ s^{-1}			E_a (kcal · mol^{-1})	Microviscosity (poise)			E_a (kcal · mol^{-1})
	5°C	20°C	35°C		5°C	20°C	35°C	
5°C-acclimated goldfish (n = 5)	1.55 ± 0.17	2.73 ± 0.31	4.61 ± 0.53	6.12 ± 0.23	2.69 ± 0.24	1.61 ± 0.16	0.97 ± 0.09	5.58 ± 0.21
25°C-acclimated goldfish (n = 6)	1.43 ± 0.10	2.52 ± 0.17	4.30 ± 0.23	5.94 ± 0.14	2.84 ± 0.18	1.71 ± 0.12	1.08 ± 0.08	5.45 ± 0.23

TABLE II

FLUORESCENCE LIFETIME VALUES AT 3.5, 20 AND 35°C FOR DPH IN SARCOPLASMIC RETICULUM PREPARATIONS ISOLATED FROM THE SKELETAL MUSCLE OF 5 AND 25°C-ACCLIMATED GOLDFISH

Values (ns) represent the mean ± S.E.; n, number of preparations.

Acclimation temperature (°C)	3.5°C		20°C		35°C	
	τ phase	τ modulation	τ phase	τ modulation	τ phase	τ modulation
5 (n = 3)	9.03 ± 0.12	9.60 ± 0.14	8.18 ± 0.22	8.78 ± 0.12	7.07 ± 0.23	7.67 ± 0.18
25 (n = 3)	9.16 ± 0.08	9.78 ± 0.10	8.42 ± 0.05	8.92 ± 0.09	7.41 ± 0.13	7.92 ± 0.09

rated fatty acids. The differences between 5- and 25°C-acclimated goldfish are presented in Table IV where they are compared with similar data obtained for a brain synaptosomal preparation which, in goldfish, exhibited a clearcut partial compensation of membrane viscosity for acclimation temperature [6].

Contrary to expectations, there were greatly increased proportions of monounsaturated fatty acids in preparations from 25°C-acclimated goldfish compared to 5°C-acclimated goldfish (Table IV); these increases were compensated for mainly by opposite changes in the proportion of polyunsaturated fatty acids. The saturated fatty acids, by contrast, showed relatively small differ-

TABLE III

THE FATTY ACID COMPOSITION OF THE MAJOR PHOSPHOGLYCERIDE CLASSES OF SARCOPLASMIC RETICULUM PREPARATIONS ISOLATED FROM THE SKELETAL MUSCLE OF 5 AND 25°C-ACCLIMATED GOLDFISH

Values represent mean % weight ± standard error of the mean for n preparations.

Fatty acid	Choline phosphoglycerides		Ethanolamine phosphoglycerides		Serine/inositol phosphoglycerides	
	5°C (n = 3)	25°C (n = 3)	5°C (n = 3)	25°C (n = 3)	5°C (n = 3)	25°C (n = 3)
16 : 0	25.2 ± 0.5	26.7 ± 0.5	8.0 ± 0.4	9.5 ± 0.7	7.0 ± 2.2	5.6 ± 0.3
16 : 1	2.4 ± 0.3	5.0 ± 1.4	1.6 ± 0.5	1.7 ± 0.7	2.1 ± 0.9	2.0 ± 0.4
18 : 0	2.7 ± 0.1	3.2 ± 0.6	8.4 ± 1.0	12.1 ± 1.0	32.9 ± 0.8	31.1 ± 1.3
18 : 1	12.9 ± 1.3	23.9 ± 3.1	11.0 ± 1.2	14.4 ± 2.8	9.1 ± 0.9	15.1 ± 2.2
18 : 2w6	21.1 ± 1.8	11.8 ± 0.6	10.3 ± 0.6	5.8 ± 0.6	3.2 ± 0.8	3.3 ± 0.8
18 : 3w3	2.2 ± 0.1	1.7 ± 0.3	2.9 ± 0.2	3.0 ± 0.2	0.8 ± 0.1	0.8 ± 0.1
20 : 4w6	9.1 ± 1.6	8.3 ± 3.3	17.2 ± 1.5	14.3 ± 0.4	19.8 ± 1.1	18.9 ± 0.8
20 : 5w3	3.2 ± 0.3	1.9 ± 0.5	3.7 ± 0.2	1.7 ± 0.2	2.6 ± 0.6	1.4 ± 0.3
22 : 5w3	1.8 ± 0.2	1.1 ± 0.3	3.4 ± 0.4	2.2 ± 0.3	3.1 ± 0.4	2.3 ± 0.3
22 : 6w3	11.9 ± 1.2	9.3 ± 2.3	22.9 ± 1.9	22.3 ± 3.1	11.3 ± 1.2	9.2 ± 0.9
Others	7.6	6.7	10.6	12.8	7.9	10.4
Total saturated	29.8 ± 0.4	31.6 ± 0.4	17.7 ± 1.2	22.7 ± 1.3	41.4 ± 2.5	38.0 ± 0.8
Total monounsaturated	15.5 ± 1.5	28.0 ± 4.3	12.9 ± 1.6	16.9 ± 3.4	11.3 ± 1.4	17.4 ± 2.7
Total polyunsaturated	54.6 ± 1.8	40.3 ± 4.7	68.8 ± 2.6	59.8 ± 3.2	46.9 ± 3.2	44.4 ± 2.1
Unknown	0.1	0.1	0.5	0.6	0.5	0.2

TABLE IV

SUMMARY TABLE TO ILLUSTRATE THE DIFFERENCE IN FATTY ACID COMPOSITION BETWEEN 5 AND 25°C-ACCLIMATED GOLDFISH, FOR BOTH MUSCLE SARCOPLASMIC RETICULUM AND BRAIN SYNAPTOSOME PREPARATIONS

See text for discussion.

Phosphoglyceride fraction	Fatty acid fraction	Muscle sarcoplasmic reticulum	Brain synaptosomes **
Choline phosphoglycerides	Saturated	+1.8 *	+5.3 *
	Monounsaturated	+12.5	—1.6
	Polyunsaturated	—14.3	—3.6
Ethanolamine phoshoglyerides	Saturated	+5.0	+8.3
	Monounsaturated	+4.0	—5.8
	Polyunsaturated	—9.0	—2.4
Serine/inositol phosphoglycerides	Saturated	—3.4	+7.3
	Monounsaturated	+6.1	—5.8
	Polyunsaturated	—2.5	—1.4

* Values represent the difference between the value for 25°C-acclimated goldfish and the corresponding value for 5°C-acclimated goldfish.

** Synaptosomes data from ref. 6.

ences; in the choline phosphoglycerides which account for over 50% of the polar lipids of sarcoplasmic reticulum membranes [13], the proportion of saturated fatty acids in the 25°C-acclimated goldfish was only 1.8% greater than in the 5°C-acclimated goldfish. In serine and inositol phosphoglycerides there was a slightly greater proportion of saturated fatty acids in the 5°C-acclimated goldfish.

In brain synaptosomal preparations (Table IV) the situation was quite different. In all phosphoglyceride fractions the proportion of saturated fatty acids increased, and the proportions of monounsaturated and polyunsaturated fatty acids decreased at the higher acclimation temperature. In addition, differences in the proportion of saturated fatty acids between 5- and 25°C-acclimated goldfish were substantially greater than in the sarcoplasmic reticulum preparations.

Comparative studies. Earlier studies on the brain synaptosomal preparations from fish obtained from the Bering Sea and the desert springs of Death Valley, California, and from rat, demonstrated considerable differences in lipid composition and membrane viscosity which appeared to be related to their respective brain temperatures [9]. A similar analysis was performed for the sarcoplasmic reticulum preparations of the same species to determine if the apparent lack of homeoviscous response was peculiar to goldfish.

The polarization/temperature graphs for the three species were very similar. The results together with some of those described earlier for the differently acclimated goldfish, are summarized in Fig. 3 as a graph of polarization (measured at 25°C) for each preparation plotted against cellular temperature. The values for Arctic Sculpin, Desert Pupfish and rat were very similar to those of 5-, 15- and 25°C-acclimated goldfish. Had there been any systematic differences between the preparations which were related to temperature adaptation,

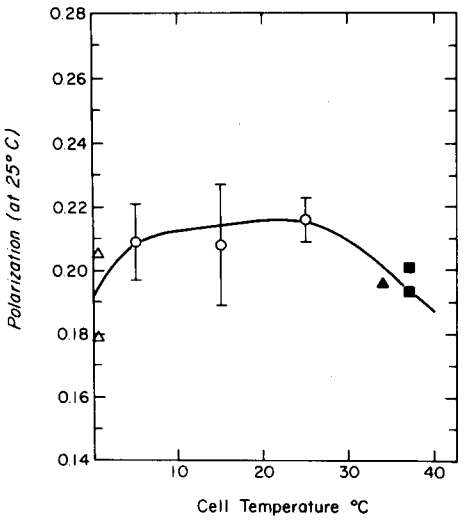


Fig. 3. Effect of seasonal acclimation and evolutionary adaptation to different thermal environments upon the membrane fluidity of muscle sarcoplasmic reticulum preparations. Membrane fluidity is expressed as polarization measured at 25°C. Points for Arctic Sculpin (0.5°C, △), Desert Pupfish (34°C, ▲) and rat (37°C, ■) represent an individual preparation. Points with standard error bars (○) are for goldfish acclimated to 5°C (n = 5), 15°C (n = 3) and 25°C (n = 6).

the polarization/cellular temperature plot (Fig. 3) would have had a positive slope.

Despite the apparent lack of differences in membrane viscosity there were considerable differences in the composition of fatty acid moieties in the sarcoplasmic reticulum preparations isolated from the various fish species and from rat (Table V). In all phosphoglyceride fractions the saturated fatty acids comprised higher proportions in animals with higher acclimation or body (i.e., cellular) temperature and this was compensated by correspondingly smaller proportions of mono- and polyunsaturated fatty acids. Comparing the results for 5 and 25°C-acclimated goldfish (Table III) with those obtained for the other fish species and for rat (Table V), there was an uninterrupted increase in the proportion of saturated fatty acids with higher cellular temperature in both choline and ethanolamine phosphoglycerides. Thus, differences between species tend to be in the expected direction. The correlation was less good in the serine and inositol phosphoglyceride fraction, a feature which was noted in other studies with brain synaptosomes [6]. The correlation of an unsaturation index of the phosphoglyceride fractions of sarcoplasmic reticulum membranes with cell temperature was poor (Table VI). For example, the unsaturation index for the choline phosphoglyceride fraction of rat and 25°C-acclimated goldfish were similar despite a difference of 12°C in cellular temperature. In addition, the value for the Desert Pupfish exceeded those obtained for the 5- and 25°C-acclimated goldfish and rat. In all phosphoglyceride fractions the Arctic Sculpin possessed the highest unsaturation index.

TABLE V

THE FATTY ACID COMPOSITION OF THE MAJOR PHOSPHOGLYCERIDE CLASSES OF MUSCLE SARCOPLASMIC RETICULUM PREPARATIONS OF THE ARCTIC SCULPIN, DESERT PUPFISH AND RAT

	Choline phosphoglycerides			Ethanolamine phosphoglycerides			Serine/inositol phosphoglycerides		
	0°C Arctic Sculpin (n = 1)	34°C Desert Puspfish (n = 1)	37°C Rat (n = 3)	0°C Arctic Sculpin (n = 1)	34°C Desert Pupfish (n = 1)	37°C Rat (n = 3)	0°C Arctic Sculpin (n = 1)	34°C Desert Pupfish (n = 1)	37°C Rat (n = 3)
16 : 0	21.8 *	27.0	36.5 ± 1.2	5.1	11.2	16.0 ± 4.1	4.3	9.0	4.7 ± 0.8
16 : 1	4.8	2.8	0.7 ± 0.4	2.9	4.2	1.1 ± 0.6	2.2	4.6	1.1 ± 0.3
18 : 0	1.2	3.9	7.7 ± 0.2	4.0	11.0	23.1 ± 4.3	28.0	21.9	42.7 ± 1.7
18 : 1	12.8	12.1	7.8 ± 0.2	20.9	14.5	5.9 ± 0.2	10.9	18.0	5.7 ± 0.5
18 : 2w6	1.1	7.8	17.0 ± 1.0	1.8	4.6	5.2 ± 0.7	1.1	4.0	1.8 ± 0.1
18 : 3w3	2.0	1.3	0.5 ± 0.1	7.2	2.7	0.3 ± 0.1	3.8	1.3	0.2 ± 0.1
20 : 4w6	2.8	7.8	14.6 ± 0.3	3.4	3.7	7.8 ± 0.3	7.9	9.1	20.1 ± 1.8
20 : 5w3	25.0	2.7	0.5 ± 0.1	14.5	1.0	0.4 ± 0.1	9.8	1.2	—
22 : 5w3	1.4	2.5	1.8 ± 0.1	1.7	2.2	3.2 ± 0.6	1.9	1.7	2.5 ± 0.1
22 : 6w3	23.9	20.2	10.0 ± 0.3	34.0	24.7	29.0 ± 2.6	23.2	10.2	8.3 ± 0.5
Others	3.3	12.1	3.0	4.5	20.3	8.1	6.9	19.3	12.8
Total saturated	23.4	34.4	45.4 ± 1.2	9.7	28.1	40.9 ± 3.2	34.7	38.3	49.4 ± 1.1
Total monounsaturated	17.7	16.0	8.7 ± 0.6	25.0	21.1	7.8 ± 0.6	14.8	25.8	7.3 ± 0.7
Total polyunsaturated	57.6	49.0	45.4 ± 0.9	64.6	48.8	49.8 ± 2.5	49.6	34.4	39.1 ± 1.7
Unknown	1.3	0.6	0.5	0.8	2.1	1.5	1.0	1.7	4.2

* Values represent mean % weight ± standard error of the mean.

TABLE VI

A COMPARISON OF THE UNSATURATION INDEX FOR THE CHOLINE, ETHANOLAMINE AND SERINE/INOSITOL PHOSPHOGLYCERIDES FOR SARCOPLASMIC RETICULUM PREPARATIONS ISOLATED FROM VARIOUS FISH SPECIES AND FOR RAT

Unsaturation index was calculated as the sum of the weight % multiplied by the number of olefinic bonds for each fatty acid in the mixture.

Phospholipid fraction	0°C Arctic Sculpin (n = 1)	5°C Goldfish (n = 3)	25°C Goldfish (n = 3)	34°C Desert Pupfish (n = 1)	37°C Rat (n = 3)
Choline phosphoglycerides	318	219	183	239	174
Ethanolamine phosphoglycerides	356	318	289	258	255
Serine/inositol phosphoglycerides	265	220	205	175	173

Discussion

The present results contrast strongly with those obtained in an earlier study of homeoviscous adaptation of goldfish synaptosomal membranes [6]. The estimated membrane fluidity of brain synaptosomal preparations proved to be highly reproducible, suggesting a precise regulation of this parameter. Acclimation of goldfish to lower environmental temperatures resulted in a more fluid hydrophobic environment at all measurement temperatures. This tends to offset the direct effects of the different environmental temperatures upon membrane fluidity and may function to maintain the structure and function of membranes at least partially independent of seasonal changes in temperature. Similar differences were observed in liposomes composed of the purified phospholipids of brain synaptosomal preparations of 5- and 25°C-acclimated goldfish, indicating that a substantial portion of this adaptive response can be accounted for by compositional changes in the membrane phospholipids. Indeed, acclimation to higher temperatures resulted in a greater proportion of saturated fatty acids in the major phospholipid classes.

The membrane fluidity of sarcoplasmic reticulum preparations proved to be somewhat variable, a factor which obscured any consistent differences between differently acclimated goldfish. Although the fatty acid composition of sarcoplasmic reticulum phosphoglycerides showed significant differences between the 5- and 25°C-acclimated goldfish, these were confined mainly to complementary changes in the relative proportions of the mono- and polyunsaturated fatty acids. In contrast to expectations, all phosphoglyceride fractions of the sarcoplasmic reticulum preparations from 25°C-acclimated goldfish possessed substantially greater proportions of monounsaturated fatty acids (principally 18 : 1) than corresponding preparations from 5°C-acclimated goldfish. The proportion of saturated fatty acids showed relatively small differences between the 5- and 25°C-acclimated goldfish. It is suggested that the effects of the increased proportion of polyunsaturated fatty acids during cold acclimation upon the dynamic structure of the sarcoplasmic reticulum membranes will be opposed by the effects of the reduced proportions of monounsaturated fatty acids, resulting in an unaltered membrane fluidity. The absence of a

viscosity adaptation in sarcoplasmic reticulum membranes can be reconciled to the 'observed' differences in their constituent fatty acid profiles, if, as before [6,14] the proportion of saturated fatty acids is considered to be of prime importance to membrane dynamic structure. The exchange of monounsaturated for polyunsaturated fatty acids or vice versa need not necessarily have as great an effect upon membrane fluidity since the greatest effects upon the physical properties of phosphoglycerides (i.e., melting point, area per molecule, force-area curves) occur with the inclusion of the first and second olefinic bond into a saturated hydrocarbon chain [3]. Successive olefinic bonds produce smaller effects upon monolayer and bilayer properties [15].

It is concluded that compared to brain synaptosomes, the sarcoplasmic reticulum membranes of goldfish have a diminished ability to regulate membrane fluidity. This difference in adaptive ability may represent a more general distinction between cell membranes and intracellular organelles. Significantly, the cellular membranes of bacteria exhibit an almost complete homeoviscous adaptation [4,7]. The lack of homeoviscous response in the sarcoplasmic reticulum of goldfish is supported to some extent by comparative studies, since there was no consistent difference in the polarization/temperature graphs of rat and the various fish species adapted over an evolutionary time-scale to diverse thermal environments. Brain synaptosomes, by contrast, exhibited a clear trend toward increased membrane fluidity in animals from colder environments which was related to differences in the fatty acid composition of the major phosphoglycerides [9].

On the basis of earlier experiments [9] one might have expected that the observed differences in fatty acid composition of the sarcoplasmic reticulum of various fish species and rat would have resulted in measurable differences in their respective polarization values. The absence of such differences indicates that in this particular membrane preparation the probe is not sensitive to small differences in fatty acid composition. Alternatively, for one reason or another, a direct comparison of the sarcoplasmic reticulum of different species is not valid and that other factors must be of major importance in determining the nature of the environment of the probe, and by inference, the dynamic structure of the hydrophobic interior of the sarcoplasmic reticulum membrane. Of particular relevance in this respect may be the presence in sarcoplasmic reticulum membranes of a proteolipid that has a marked ordering influence upon the bulk hydrocarbon environment [16–18].

Morse et al. [19] have demonstrated with electron spin resonance spectroscopy that rabbit sarcoplasmic reticulum membranes are more ordered at all measurement temperatures than those of lobster, and despite the large phylogenetic distance between these two organisms it is tempting to attribute this difference to adaptive responses for their respective thermal environments. However, a major problem in all techniques involving the use of exogenous probe molecules is the lack of information regarding their position both in the bilayer and in the plane of the membrane. The discrepancy between the present results and those of Morse et al. [19] may be the result of different binding sites for the fluorescence and spin probes. Clearly, comparative studies with other fluorescence or ESR probes, or preferably, with a non-perturbing technique, are required to resolve this issue and studies on the simpler phos-

pholipid liposome system prepared from sarcoplasmic reticulum phospholipids are currently underway.

It should also be noted, that as a description of the dynamic structure of biological membranes, the absolute values of polarization and derived quantities measured by the fluorescence polarization technique are open to some criticism, due to the complex nature of the rotational relaxation of DPH, even in simple model membrane systems [20]. However, polarization and derived quantities have greater relevance when comparing similar membranous preparations, since considerations of the nature of the relaxation process are thought to apply equally to each preparation.

The fluorescence polarization technique gives average information for all hydrophobic sites sampled by the probe molecules and for this reason it is not possible to rule out functional or structural adaptations that are associated with a small proportion of the membranous vesicles, with specific microenvironments within the membrane or in areas not sampled by the fluorophore. Changes in the fatty acid composition of those phospholipids that comprise the 'annulus' of membrane-bound proteins may affect their functional properties without any effect upon the bulk membrane fluidity. With respect to adaptations of sarcoplasmic reticulum function earlier reports are contradictory. Madeira and Antunes-Madeira [21] have described differences in the fatty acid composition of sarcoplasmic reticulum membranes of lobster and rabbit which correlate with differences in the temperatures of discontinuities in the Arrhenius plots of the associated Ca^{2+}-stimulated ATPase [22]. On the other hand, the same enzyme of freshwater crayfish acclimated to 4 or 25°C exhibited identical stabilities at high inactivating temperatures [23], specific activities, Ca^{2+} activation kinetics and ATP saturation curves over a wide range of assay temperatures [24] despite differences in the fatty acid compositon of whole muscle phosphoglycerides [5]. Becker and Willis [25] were unable to demonstrate cold adaptation of Ca^{2+}-stimulated ATPase in the sarcoplasmic reticulum of hibernating ground squirrels. Tume et al. [26] failed to observe differences in the Ca^{2+}-stimulated ATPase and calcium uptake kinetics of sarcoplasmic reticulum preparations isolated from rats fed fat-free and lipid-supplemented diets, even though the fatty acid composition of their membranes was affected. Finally, lipid substitution of the phospholipid annulus of the Ca^{2+}-stimulated ATPase with dioleoyl, dieloidoyl or 1-palmitoyl, 2-oleoyl phosphatidylcholines produced no differences in its specific activity or its temperature dependence (Warren, G.B., personal communication). These latter findings suggest that the Ca^{2+}-stimulated ATPase activity, at least, is independent of such changes in membrane lipid composition as occur during thermal acclimation of goldfish. The Ca^{2+} uptake and permeability properties have not been examined from this viewpoint.

If one accepts that sarcoplasmic reticulum function is not measurably affected by changes in the unsaturation of its associated phosphoglycerides then homeoviscous adaptation would serve no useful purpose. The same would apply if certain sarcoplasmic reticulum functions were not rate limiting to muscle contraction or relaxation [27]. Thus, taken at face value, the present results suggest an economy of adaptive 'effort' in higher animals, with homeoviscous adaptation being demonstrable only in those cases where some selective

benefit is ensured. A similar case may be exhibited by hibernating mammals which show no evidence of cold adaptation of membrane fluidity in either brain synaptosomes or in kidney microsomes (Cossins and Wilkinson, unpublished observations). In this case, the necessity for normal synaptic function at the high normal body temperatures during the brief arousal periods that may frequently occur during hibernation may be of greater importance than adaptation of synaptic function to the reduced temperatures that are characteristic of hibernation. However, in those poikilothermic vertebrates, which of necessity remain active despite large seasonal fluctuations in environmental temperature, some compensation of synaptic function to temperature is necessary.

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