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Evolutionary adaptation of membranes to temperature

(membrane fluidity/synaptosomes/thermal tolerance/fatty acids/phospholipids)

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ABSTRACT **The "fluidity" of brain synaptosomal membrane preparations of arctic and hot-springs fish species, two temperate water fish species acclimated to different seasonal temperatures, and two mammals was estimated using the fluorescence polarization technique. At all measurement temperatures, the fluidity decreased in the order: arctic sculpin, 5°-acclimated goldfish, 25°-acclimated goldfish, desert pupfish, and rat. This correlated with increasing adaptation or body (i.e., cellular) temperatures of 0°, 5°, 25°, 34°, and 37° and suggested a partial compensation of membrane fluidity for environmental temperature that occurs over the evolutionary time period as well as during laboratory (seasonal) acclimation. Evolutionary adaptation of relatively stenothermal species to constant thermal environments resulted in a more complete compensation than laboratory (seasonal) acclimation. Each compensation is accompanied by differences in the saturation of membrane phosphoglycerides. At increased cellular temperatures the proportion of saturated fatty acids increased and the unsaturation index decreased; the correlation between these indices and the measured expression of membrane dynamic structure was highly significant. It is concluded that the homeoviscous compensation of synaptic membrane function is an important component of temperature adaptation.**

Biological membranes resemble a two-dimensional, hydrophobic fluid whose dynamic nature has important consequences for a number of membrane-associated functional properties (1). A variety of organisms possess the ability to modulate the fluidity of their constituent cellular membranes in compensation for the direct effects of altered environmental temperature, a phenomenon termed "homeoviscous adaptation" (2). In *Tetrahymena* (3) and the synaptosomal membranes of the goldfish *Carassius auratus* (4), partial compensation is achieved after laboratory acclimation at different temperatures, but it is somewhat less than that required to maintain a constant "fluidity" at all environmental temperatures; partial compensation may be associated with the eurythermal properties of these animals. Bacterial membranes show a complete compensation (2, 5).

Fishes that inhabit relatively constant thermal environments, particularly such extremes as polar seas or thermal springs, may be expected to exhibit a more complete homeoviscous adaptation because for them the maintenance of a eurythermal ability has no evolutionary significance. Indeed, stenothermal species often exhibit a high degree of adaptation to their respective environments such that they perish at temperatures only slightly removed from normal (6). To test the hypothesis of homeoviscous adaptation, we present here comparative studies of the fluidity and biochemical composition of synaptosomal membranes isolated from fish that live in arctic or hot-springs environments, other fish adapted to a wide range of temperatures, and rat and hamster. Synaptosomal membranes were used because resistance adaptation to temperature in fishes is in large part due to maintenance of synaptic function after acclimation to hot or cold temperatures (7).

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MATERIALS AND METHODS

Animals. Arctic sculpin [*Myoxocephalus verrucosus* (Bean), tentative identification, 27–30 cm length] were caught near St. George Island in the Bering Sea at approximately −0.3° and maintained in the laboratory in artificial sea water at 0 ± 1°. Desert pupfish (*Cyprinodon nevadensis*, 3–4 cm) were reared at approximately 28° from stocks originally obtained from Saratoga Springs, Death Valley National Monument, California. On arrival in Illinois they were kept at 28° for 2 days and slowly warmed over a 4-day period to 34 ± 0.5°, where they were kept for 7 days before sacrifice. Goldfish were obtained commercially and acclimated for at least 21 days, some at 5°, others at 25° (4). Green sunfish–bluegill hybrids (*Lepomis sp.*) were obtained from Illinois ponds and were acclimated to 5° or to 25° for at least 21 days. Rats and hamsters were from laboratory stock.

Preparation of Brain Synaptosomes. Brain synaptosomes were prepared by differential and discontinuous sucrose gradient centrifugation as described previously (4).

Membrane Fluidity. The fluidity of brain synaptosomal membranes was estimated using the fluorescence polarization technique with 1,6-diphenyl-1,3,5-hexatriene (Aldrich "puriss" grade) as fluorescence probe (4, 8, 9). Results are expressed as polarization of fluorescence; an increased value indicating a reduced rate of probe motion and by inference a more restrictive hydrophobic environment (4).

Fatty Acid Analysis. Synaptosomal lipids were extracted, the major phosphoglyceride fractions were purified by two-dimensional thin-layer chromatography, and their constituent fatty acids were analyzed by gas–liquid chromatography, all as described previously (4).

RESULTS

Arrhenius plots of polarization for brain synaptosome preparations of the arctic sculpin, 5°- and 25°-acclimated goldfish, desert pupfish, and rat are presented in Fig. 1. The experiments on the arctic sculpin and desert pupfish were performed simultaneously with analyses of 5°- and 25°-acclimated goldfish, respectively, in an effort to reduce the effects of minor differences of preparative technique upon the comparison. The data for rat synaptosomes have been reported previously (4) and have been included for comparative purposes. The curves clearly show (*a*) an increased value of polarization (and by inference of membrane order) with reduced measurement temperature and (*b*) a shift of the curves upwards and to the left with higher acclimation, habitat, or body (i.e., cellular)

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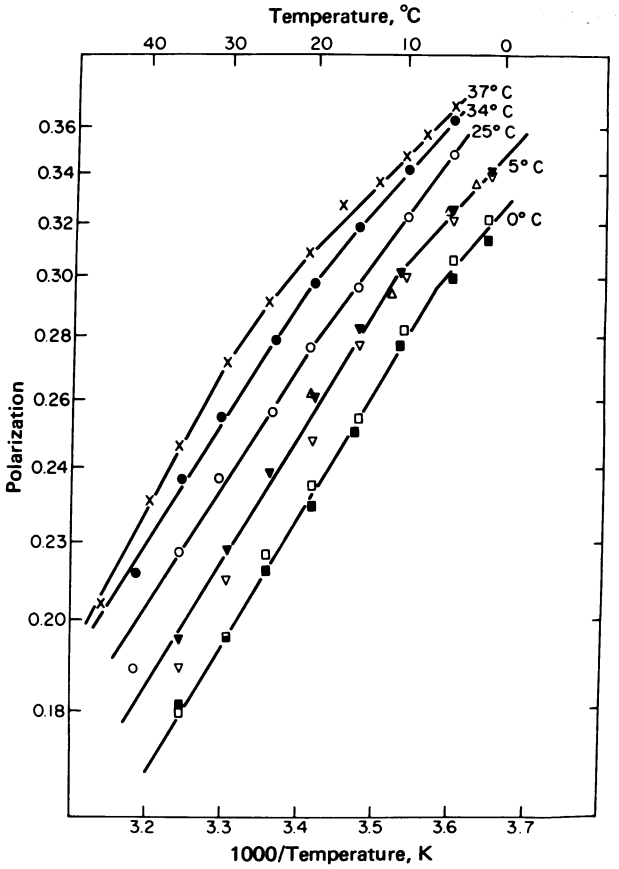

FIG. 1. Arrhenius plots of polarization for diphenylhexatriene incorporated into synaptosomal membrane preparations of various fish species and rat. Arctic sculpin (0°, □, ■); 5°-acclimated goldfish (5°, ▼, △, ▽); 25°-acclimated goldfish (25°, ○); desert pupfish (34°, ●); rat (37°, ×). Each symbol represents a separate preparation.

temperature. Thus, over the entire temperature range, synaptosomal membranes of the arctic sculpin were more fluid than those of 5°-acclimated goldfish, and the synaptosomal membranes of the desert pupfish had a fluidity between those of 25°-acclimated goldfish and the rat.

These results are summarized in Fig. 2, together with those obtained earlier for 5°-, 15°-, and 25°-acclimated goldfish (4), as a plot of polarization value for each animal at its respective cellular temperature (i.e., acclimated or body temperature) against its cellular temperature. Thermal acclimation of goldfish and green sunfish–bluegill hybrids resulted in a partial compensation of synaptosomal membrane fluidity for changes in environmental temperature, because the estimated membrane fluidity (as expressed by polarization values) was not identical at each of the various acclimation temperatures but was relatively constant at temperature extremes (Fig. 2). The polarization values for the arctic sculpin at 0° were slightly lower than the values for the 5°-acclimated goldfish and green sunfish–bluegill hybrid measured at 5°. Similarly, the values for the desert pupfish at 34° approached those of rat and hamster at 37°.

The fatty acid compositions of the major phosphoglyceride classes of synaptosomal preparations isolated from the synaptic membranes of arctic sculpin and desert pupfish are presented in Table 1. The corresponding data for 5°- and 25°-acclimated goldfish and for rat have been reported previously (4). The data for all of these preparations have been summarized in Table 2 as two indices, the ratio of saturated to unsaturated fatty acids (saturation ratio), and as an unsaturation index that gives a

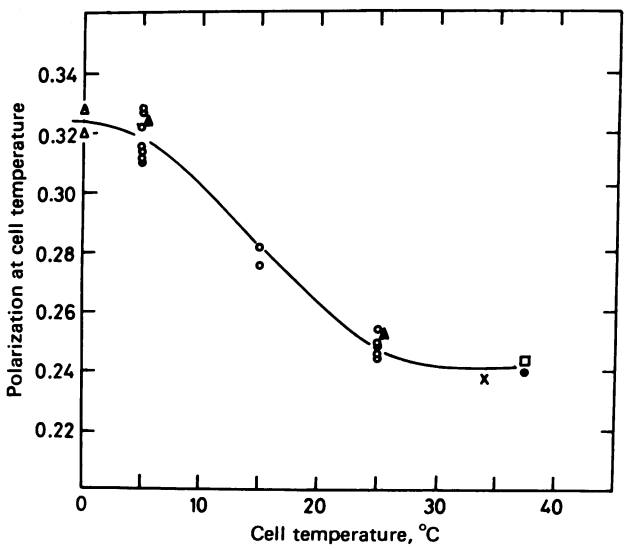

FIG. 2. Effect of adaptation or acclimation at different temperatures upon membrane viscosity expressed as polarization measured at their respective acclimation, environmental, or body (i.e., cellular) temperatures. Arctic sculpin (△), goldfish (○), green sunfish–bluegill hybrid (▲), desert pupfish (×), rat (●), and hamster (□). Each point represents an individual animal.

relative estimate of the number of olefinic bonds in each phospholipid class. Despite differences in the dietary regimen of the various organisms and perhaps in desaturating ability, there was an unmistakable trend towards increased fatty acid unsaturation with the lower cell temperatures, particularly in the choline phosphoglycerides, where there was an increased proportion of unsaturated fatty acids as well as an increase in the average number of olefinic bonds per unsaturated fatty acid. In the ethanolamine phosphoglyceride fraction the saturation ratio decreased with lower cell temperature, but this was not associated with consistent effects upon the unsaturation index. Fig. 3 presents the correlation between membrane fluidity as expressed by polarization with the saturation ratio for ethanolamine phosphoglyceride and choline phosphoglyceride. In all phospholipid classes the saturation ratio exhibited a more significant and extensive correlation with polarization than did the unsaturation index, as shown in earlier observations (10).

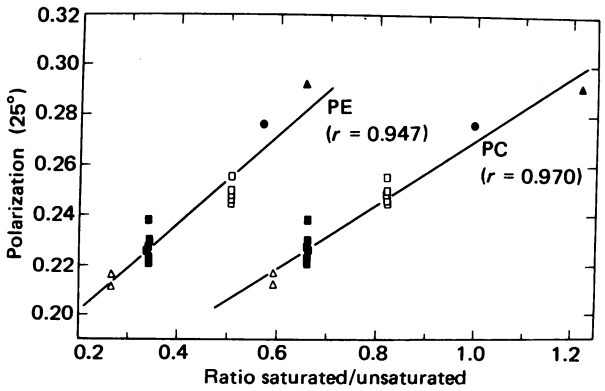

FIG. 3. Relationship between viscosity of synaptosomal membranes of various fish species and rat, and the ratio of saturated to unsaturated fatty acids for choline phosphoglycerides (PC) and ethanolamine phosphoglycerides (PE). Each point represents an individual animal. Membrane viscosity is expressed as polarization of diphenylhexatriene measured at 25° and fatty acid data are from Table 2. Arctic sculpin (△), goldfish acclimated at 5° (■), goldfish from 25° (□), desert pupfish (●), rat (▲).

Table 1. Fatty acid composition (weight %) of the major phosphoglyceride fractions isolated from the synaptosomal membranes of the arctic sculpin and desert pupfish

	Choline phosphoglycerides		Ethanolamine phosphoglycerides		Serine/inositol phosphoglycerides	
Fatty acid	Arctic sculpin	Desert pupfish	Arctic sculpin	Desert pupfish	Arctic sculpin	Desert pupfish
16:0	31.08	33.14	11.63	11.92	11.39	16.98
16:1ω9	6.37	5.61	2.97	3.83	3.93	7.51
18:0	4.09	12.26	7.31	18.79	16.39	16.99
18:1ω9	19.77	27.36	18.86	15.12	16.55	21.64
18:2ω6	0.93	1.76	1.00	1.96	1.13	1.44
18:3ω3	0.34	0.64	1.31	1.31	1.30	1.73
20:4ω6	1.32	1.63	1.64	2.73	3.17	3.10
20:5ω3	4.57	0.29	7.20	—	4.32	—
22:5ω6	0.28	0.90	0.11	1.96	0.28	3.57
22:6ω3	25.78	6.97	42.30	27.03	29.55	14.61
Others	5.19	9.29	5.68	14.96	11.78	12.04
Saturated	36.85	49.29	20.65	34.47	31.32	37.44
Monounsaturated	26.69	34.47	22.48	22.55	22.17	31.35
Polyunsaturated	35.41	15.31	56.88	38.18	43.55	29.41
Unidentified	0.77	0.78	—	4.41	2.75	1.61

Synaptosomal lipids were extracted and purified and fatty acid methyl esters were prepared as described in ref. 1. The fatty acid composition of purified phosphoglycerides was analyzed and quantitated using a Hewlett–Packard 5830A gas chromatograph with a 1500- × 2-mm (inside diameter) glass column packed with 10% SP-2340 on Chromosorb W AW (Supelco, Bellefonte, PA). Column temperature was 180° and carrier gas flow rate was 25 ml/min.

DISCUSSION

Laboratory acclimation (and presumably seasonal acclimation) of the eurythermal fish species, the goldfish and the green sunfish–bluegill hybrids, has previously been shown to lead to a modification of their synaptosomal membrane fluidity–temperature curves, a response which is thought to result in a partial compensation of membrane structure for the direct effects of a changed environmental temperature (4). The time course during warm and cold acclimation of homeoviscous changes closely parallels changes in behavior under thermal stress and changes in fatty acid composition (10). Fish species normally living in arctic or hot-springs water and homeotherms appear to have achieved a relatively complete adaptation to their respective environmental temperatures. However, the fact that the polarization values for the arctic sculpin at 0° differ from the corresponding values for the desert pupfish and rat at their respective environmental temperatures indicates that this adaptation cannot be considered "ideal" or "perfect" in the sense proposed by Precht (11).

It is possible that the necessity of eurythermal fish species to tolerate and adapt to relatively large seasonal fluctuations in environmental temperature has placed a constraint upon their ability to modulate membrane dynamic structure for a change of environmental temperature and hence may be viewed as the price that must be paid for a eurythermal capacity. By contrast, species that exist in relatively unchanging thermal environments can permit specialization of their membranous structures to particular temperatures without having to exhibit seasonal flexibility, resulting in a more complete adaptation of membrane structure to that temperature.

However, incomplete homeoviscous adaptation during either seasonal or evolutionary adaptation does not necessarily imply a partial compensation of membrane-associated functions that are affected by membrane fluidity. For example, if the "optimal" state towards which the adaptation is directed changes with environmental temperature, the compensation observed may be considered complete in a functional sense. Alternatively, the occurrence of compensatory mechanisms in addition to the homeoviscous response may result in a more complete overall

Table 2. Summary of the fatty acid composition of brain synaptosomes isolated from various fish species and the rat

	Phospho-glyceride	r	Arctic sculpin, 0°	Goldfish 5°	Goldfish 25°	Desert pupfish, 34°	Rat, 37°
Ratio saturated to unsaturated fatty acids	Choline	0.970	0.593	0.659	0.817	0.990	1.218
	Ethanolamine	0.947	0.260	0.340	0.506	0.568	0.651
	Serine/inositol	0.811	0.477	0.459	0.633	0.616	0.664
Unsaturation index*	Choline	0.938	220.8	172.5	149.5	102.8	98.5
	Ethanolamine	0.830	333.1	317.2	316.4	227.6	250.5
	Serine/inositol	0.558	254.8	294.3	284.8	177.0	233.4
Average olefinic bonds per unsaturated fatty acid†	Choline	0.875	3.56	2.86	2.71	2.07	2.20
	Ethanolamine	0.008	4.24	4.25	4.77	3.75	4.15
	Serine/inositol	0.233	3.88	4.30	4.65	2.96	3.88

r, Correlation coefficient for the dependence of membrane fluidity as expressed by polarization at 25° upon the various indices of fatty acid composition for each phosphoglyceride class.

* Calculated as sum of weight % multiplied by number of olefinic bonds for each fatty acid in the mixture.

† Calculated as unsaturation index divided by proportion of unsaturated fatty acids.

compensation of body functions than may be seen using polarization alone. Of interest in this respect is the recent demonstration of increases of up to 50% in the concentration of cytochrome *c* (12) and cytochrome oxidase (13) and perhaps other enzymes involved in oxidative phosphorylation. Thus the overall activity of these enzymes is increased during cold acclimation or adaptation not only by the reduced constraint imposed by the more fluid phospholipid environment but also by the increased number of active enzyme molecules. Until more is known about the precise functional compensation observed during both seasonal acclimation and evolutionary adaptation to different environmental temperatures, it is not possible to ascertain the relative degrees of success of the homeoviscous adaptation observed over the two time courses.

The close agreement between the polarization values obtained for synaptosomes of differently acclimated goldfish and green sunfish–bluegill hybrids (Fig. 2) and between the values for the desert pupfish and rat and hamster at their respective environmental or body temperatures, suggests a general relationship between cellular temperature and synaptosomal membrane fluidity which crosses broad phylogenetic boundaries. The excellent correlation between the estimated fluidity of the synaptosomal membranes of different species and the fatty acid composition of their respective phosphoglycerides (Fig. 3 and Table 2) indicates that membrane fluidity is principally influenced by the fatty acid composition of its phosphoglycerides. The differences in the synaptosomal fatty acid composition of the various species is therefore related to their respective cellular temperatures despite differences in the composition of dietary lipids, idiosyncracies of lipid metabolism, and other structural modifications that may have arisen during evolutionary development.

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