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Correlations between Behavioral Temperature Adaptations of Goldfish and the Viscosity and Fatty Acid Composition of Their Synaptic Membranes

Andrew R. Cossins*, Michael J. Friedlander**, and C. Ladd Prosser

Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801, USA

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Summary. 1. Goldfish acclimated either to 5 °C or to 25 °C were transferred to the opposite temperature and the changes in behavioral resistance to high temperature, the fluidity and fatty acid composition of isolated synaptosomal membranes were followed during acclimation to the new temperature with the purpose of establishing some correlation.

2. In 25 °C-acclimated goldfish, hyperexcitability was induced at 34.5 °C, loss of equilibrium at 37.6 °C and coma at 39.0 °C. In 5 °C-acclimated goldfish the corresponding temperatures were 29.2 °C, 32.0 °C and 33.0 °C. Time to attain 75% of the final acclimated state after transfer was approximately 4 days at 25 °C and 28 days at 5 °C.

3. Fluidity of synaptosomal membranes isolated from goldfish brains was estimated by use of the fluorescence polarization technique. Membrane viscosity decreased during acclimation to 5 °C, but increased during acclimation to 25 °C. The early stages of the transitions differed in time course from behavioral resistance acclimation but times to reach the new acclimated state were similar.

4. Fatty acid composition of synaptosomal phospholipids showed increased unsaturation during cold-acclimation and decreased unsaturation during warm-acclimation.

5. It is concluded that during acclimation, behavior shows changes in resistance to heat which are related to synaptic block. These are correlated in direction and overall time course with viscosity of synaptosomes as dictated by changes in the saturation of membrane phospholipids.

Introduction

At extreme temperatures in the biological range, functions of central nervous systems fail in a predictable and readily reversible sequence. A series of behav-

* Present address: A.R.C. Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, U.K.
** Present address: University of Virginia School of Medicine, Department of Physiology, Charlottesville, Virginia 22901, USA

Abbreviations: PC = Choline phosphoglycerides, PE = Ethanolamine phosphoglycerides, PS = Serine and inositol phosphoglycerides

ioral malfunctions have been described in goldfish as brain temperature approaches high or low thermal extremes; the times and temperatures at which these occur can be modified by thermal acclimation in an apparently adaptive manner (Friedlander et al., 1976). Block of synaptic transmission is significantly more sensitive to heating and cooling than is cessation of axon conduction (Prosser, 1973; Lagerspetz, 1974), strongly implicating synapses in behavioral defects in cold or heat. Inhibitory synapses appear to be more sensitive than excitatory synapses in some parts of the central nervous system (Friedlander et al., 1976). At neuromuscular junctions where pre- and postsynaptic events can be examined separately, moderate heating has suppressive effects at both sites (White, 1976) but with cooling, the more sensitive functions are associated with the postsynaptic complex (Jensen, 1972). The molecular bases of these thermally-induced synaptic malfunctions are unknown but Bowler and his colleagues (Bowler et al., 1973; Gladwell, 1975; and Gladwell et al., 1975) have provided evidence that changes in the permeability of excitable membranes and the stability of lipoprotein complexes may be involved in heat death and in resistance adaptation of poikilotherms.

Changes in lipid composition of goldfish brain and of brain synaptosomal preparations during thermal acclimation are well documented (Roots, 1968; Roots and Johnston, 1968; Driedzic et al., 1976; Cossins, 1977). Since synaptic events are essentially membrane-associated, there is good reason to expect that changes in lipid composition of membranes may play some role in both sensitivity to synaptic block and behavioral resistance adaptation of goldfish. Acclimation to colder temperatures generally results in an increased proportion of unsaturated to saturated fatty acids in membrane phospholipids (reviewed in Hazel and Prosser, 1974) and this has been correlated with the partial compensation for the viscosity change of synaptosomal membranes (Cossins, 1977). At any particular temperature of measurement, the bulk viscosity of the hydrophobic portion of the synaptosomal membranes isolated from warm-acclimated goldfish is significantly higher than that of cold-acclimated goldfish (Cossins, 1977). Thus synaptic functions which may be influenced by membrane viscosity are disrupted at higher temperatures in warm-acclimated animals than in cold-acclimated animals (Friedlander et al., 1976).

Further evidence in support of a causal relationship between thermal block of certain behavioral responses, the thermodynamic state and lipid composition of synaptic membranes may be obtained from a comparison of the time taken for these parameters to change during acclimation to new environmental temperatures. In this communication we have compared the time course of adaptation of these parameters during reacclimation of 5 °C-acclimated goldfish to 25 °C and vice versa.

Methods

1. Acclimation and Treatment of Goldfish. Common goldfish (12–15 cm, *Carassius auratus*) were obtained from a commercial source and were maintained for 7 days in a 15 ± 1 °C constant temperature room in 8-gallon aquaria filled with unchlorinated well-water. Aquaria were constantly aerated

and filtered. Goldfish were subsequently transferred to their respective acclimation temperatures by moving the fish in 15 °C water into constant temperature rooms at 5 ± 1 °C or 25 ± 1 °C. Equilibration of aquarium temperature to the acclimation temperature occurred over a period of 12–15 h. A photoperiod of 12L:12D with artificial dawn and dusk were provided by incandescent bulbs operated by a Conviron Sunrise-Sunset Simulator. Fish at 25 °C were fed twice daily, at 15 °C once daily, and at 5 °C on alternate days, with Warley's Conditioned Goldfish Food. After a minimum of 21 days, the animals were assumed to have reached their respective acclimated states. Groups of acclimated goldfish were transferred at various times over a period of two months to the opposite acclimation temperature and measurements and analyses were performed over a 2–3 day period at the termination of the experiment. Thus it was possible to have fish acclimated at 5 °C or 25 °C for different periods of time and ready for measurement on the same day. Those animals transferred from 5 °C to 25 °C were placed with their 5 °C water directly in the 25 °C constant temperature room, while animals transferred from 25 °C to 5 °C were left with their 25 °C water at 15 °C for a 24-h sojourn prior to moving them to 5 °C. Direct transfer of fish from 25 °C to 5 °C resulted in an unacceptable mortality.

2. Behavioral Observations. For behavioral testing, animals in groups of 3 were transferred to a holding tank at room temperature (22 °C), where the following observations were made: spontaneous swimming movements, breathing rate, responses to a prod on the flank with a plastic rod, righting response when turned over with a rod. The experimenter did not know the acclimation history of the animals. After exactly 30 min at 22 °C the fish were transferred to an adjacent test tank at 22 °C. Temperature of the water in the test tank was increased with a Lauda Super K-2/R thermostated water bath at 0.33 °C/min. Test tank temperature was monitored with a precision mercury thermometer to within 0.1 °C. Rapid thermal equilibration of the water was assured by continuous vigorous aeration and water bath efflux. Behavioral endpoints were defined as follows: hyperexcitability – prolonged bursts of swimming, sometimes spontaneous and sometimes initiated by a single stroke on the flank; loss of equilibrium – failure to right after being turned over with a plastic rod; coma – no detectable breathing movements for 15 s, fish floating motionless. After coma was established, each animal was rapidly transferred back to the 22 °C holding tank and allowed to recover. Shortly after complete recovery (5 min) the animals were sacrificed for isolation of the brain synaptosomal preparation.

3. Isolation of Brain Synaptosome Preparation. Goldfish were sacrificed by decapitation immediately after recovery from heat coma and a membrane preparation rich in synaptosomes was isolated using a discontinuous sucrose gradient as described previously (Cossins, 1977).

4. Estimation of Membrane Viscosity. When plane polarized light falls upon a group of molecules, those with their absorption oscillators oriented parallel to the plane of polarized light will be preferentially excited. Similarly, fluorescent light will be polarized parallel to the emission oscillator of the fluorescent molecule. In the case of a fluorophore (fluoroescent molecule) which shows no molecular rotation about the axes of the absorption and emission oscillators, the emitted light will show maximal degree of polarization. Molecular rotation of the fluorophore causes a decrease in the polarization of the fluorescent light; the value of polarization is, therefore, a convenient parameter for estimating molecular rotational motion of the fluorophore. In the present experiments, 1,6-diphenyl hexatriene (DPH), a hydrophobic molecule which preferentially partitions into the hydrophobic phase of the biological membrane, was used. Its rotational characteristics are dependent upon the molecular motion of neighboring lipid molecules and, therefore, provide an indirect index of the molecular motion of the lipids and a convenient technique for following changes in the fluidity of the membrane interior; a decreased value of polarization indicates an increased rate of rotation of the probe and therefore a less viscous membranous environment. For a more detailed description of the concepts and techniques, see Pesce et al. (1971) and Shinitzky et al. (1971).

Synaptosomes were labelled with DPH (Aldrich 'puriss' grade) and the polarization of fluorescence was measured at 25 ± 0.5 °C as described previously (Cossins, 1977). Briefly, the fluorophore was excited at 357 nm with an intense beam of vertically polarized light and the emitted light was simultaneously detected in two cross-polarized channels mounted at right angles to the incident

light. Steady-state polarization of the fluorescent light was defined as $p = \frac{I_{II} - I_I}{I_{II} + I_I}$ where I_{II} and I_I represent the intensity of emitted light detected through polarizers oriented parallel or perpendicular to the plane of polarization of the incident light, respectively.

5. Analysis of Synaptosomal Fatty Acids. A total lipid fraction of pooled synaptosomal membranes was extracted as described previously (Cossins, 1977). Major phospholipid classes were separated by one-dimensional thin layer chromatography on 0.5 mm chromatoplates using silica gel H (Merck, Darmstadt, West Germany) slurried in water. Plates were air-dried and activated at 100 °C for 1 h prior to use. Chromatoplates were developed with chloroform:methanol:7 M ammonia (230:90:15, v/v/v) and the separated lipids were located on the chromatoplate with 0.005% aqueous rhodamine 6G spray reagent (Cossins, 1976). Three phospholipid fractions were eluted from the silica gel as described previously (Cossins, 1977), corresponding to choline phosphoglycerides, ethanolamine phosphoglycerides and serine/inositol phosphoglycerides. The latter fraction also contained small quantities of sphingomyelin. Fatty acid methyl esters were prepared and analyzed by gas-liquid chromatography as described previously (Cossins, 1977). Replicate analyses of lipid preparations demonstrated the reproducibility of the data.

Results

Behavior. Animals from both transfer groups showed similar types of behavioral deficits upon exposure to water at high temperatures. Initially, a highly excitable state was observed, followed by loss of equilibrium and finally coma (Friedlander et al., 1976). All animals showed apparent total recovery after return to a room temperature (22 °C) tank. Breathing began within 30 s, then normal reflexes, righting and coordinated swimming returned within 2 to 10 min.

The animals which were transferred from 5 °C to 25 °C showed a rapid increase in the critical temperatures for behavioral malfunction with 75% of the total change being completed within 4 days for all behavioral parameters (Fig. 1a). At 10–15 days the animals were behaviorally indistinguishable from 25 °C acclimated goldfish. The group transferred from 25 °C to 5 °C similarly showed a progressive change in critical temperature but over a longer time course, with 75% of the total acclimation complete after 20–28 days (Fig. 1b). Between 40 and 50 days were required until these animals were clearly at the 5 °C acclimated state.

The initial slopes for the changes in the temperatures for the three behavioral parameters studied in fishes transferred from 5 °C to 25 °C were 1.25, 1.05, and 1.75 °C per day for hyperexcitability, equilibrium loss, and coma, respectively. The corresponding values for the 25 °C to 5 °C transfer group were only 0.24, 0.16, and 0.13 °C per day. The steady state temperatures for each behavioral endpoint differed by approximately 5.50 °C in each acclimation group.

Synaptosomal Viscosity and Fatty Acid Composition. The viscosity of synaptosomal membranes was estimated at 25 °C and is expressed as the polarization of a fluorescent probe introduced into the membrane preparation. Membranes

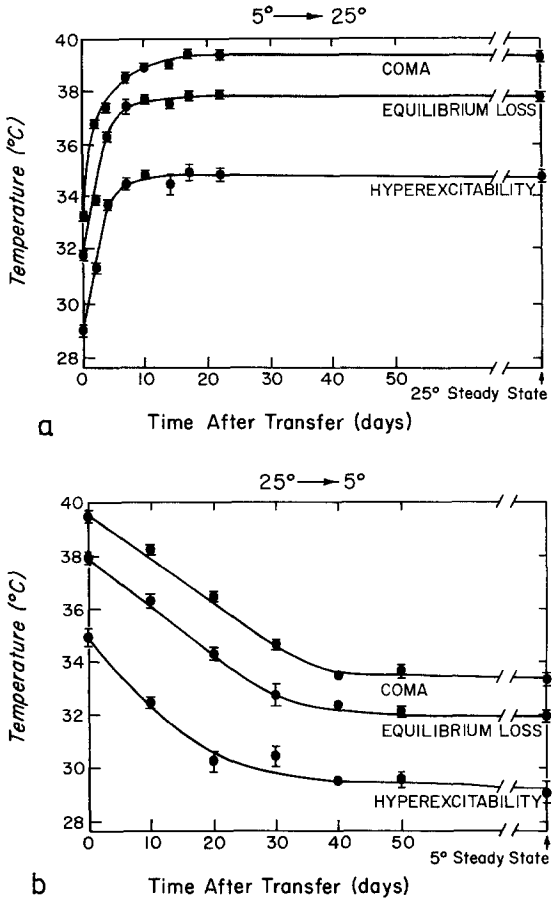

Fig. 1a and b. Time course of changes of critical high temperatures for three behavioral endpoints during acclimation (**a**) from 5 to 25 °C and (**b**) from 25 to 5 °C. In this and in other figures the error bars are ±S.E.M. In the groups transferred from 25 to 5 °C the number of fish was 5 or 6 for each data point except that the steady state group consisted of 4 and the 40-day point of 2 fish; hence no error was calculated for the 40-day point. The 40-day group was small due to loss from fungal infection during acclimation. In the groups transferred from 5 to 25 °C, all n-values were 6 fish per data point. All curves fitted visually

isolated from the brains of fish acclimated to 25 °C are more viscous, as indicated by higher polarization values (0.260 ± 0.0014), than are membranes from fish acclimated to 5 °C (polarization 0.240 ± 0.006) when both are measured at 25 °C. Low-temperature acclimated animals are thus able to compensate for the increase in viscosity due to cooling, determined in part by changes in the composition of membrane fatty acids (Cossins, 1977). The small standard errors of the mean indicate that viscosity of synaptosomal membranes is a highly conserved parameter (Cossins, 1977) and demonstrate the accuracy inherent in the fluorescence polarization technique.

Figure 2a shows the transition during acclimation of 25 °C acclimated animals to 5 °C and Figure 2b the transition from 5 °C to 25 °C. The increase

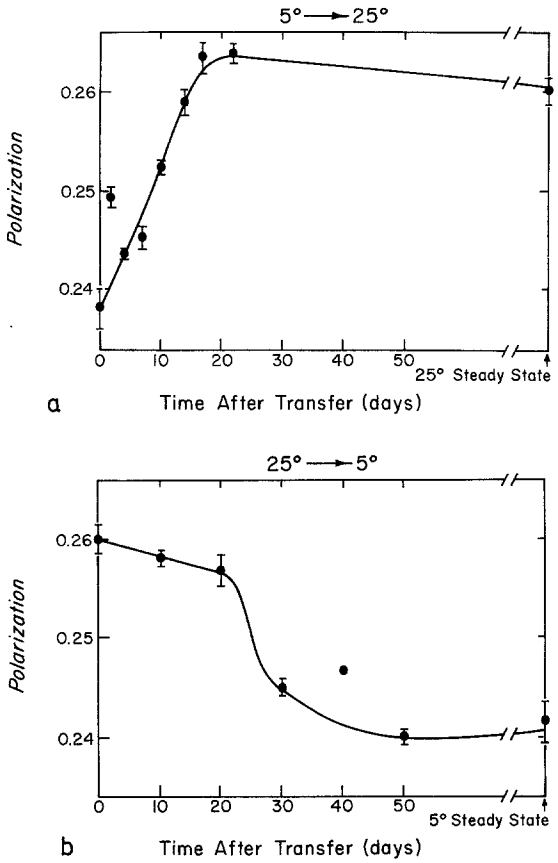

Fig. 2a and b. Time course of changes in synaptosomal membrane fluidity expressed as polarization (see text) during acclimation (**a**) from 5 to 25 °C and (**b**) from 25 to 5 °C. Error bars represent ±S.E.M.; n-values are the same as described for Figure 1

of steady-state polarization values (decrease in fluidity) on transfer from 5 to 25 °C was more direct and more rapid (15 days for 75% change) than the change from 25 to 5 °C, which showed a lag of about 20 days followed by a rapid transition (30 days to 75% change). The times to reach new steady states in the two directions were similar to the time for behavioral compensations.

The fatty acid composition of the major phospholipid classes of pooled synaptosomal membrane preparations was analyzed by gas-liquid chromatography and the results are presented graphically for the major fatty acids in Figure 3. Values are given as percent fatty acid by weight for each of three phospholipid classes: choline phosphoglycerides (PC), ethanolamine phosphoglycerides (PE) and serine/inositol phosphoglycerides including sphingomyelin (PS).

During the transition from 5 °C to 25 °C, PC showed slightly decreased proportions of 16:1 and 22:6 with an increase in the proportion of 16:0. PE showed a marked increase in the proportion of 18:0 and a corresponding

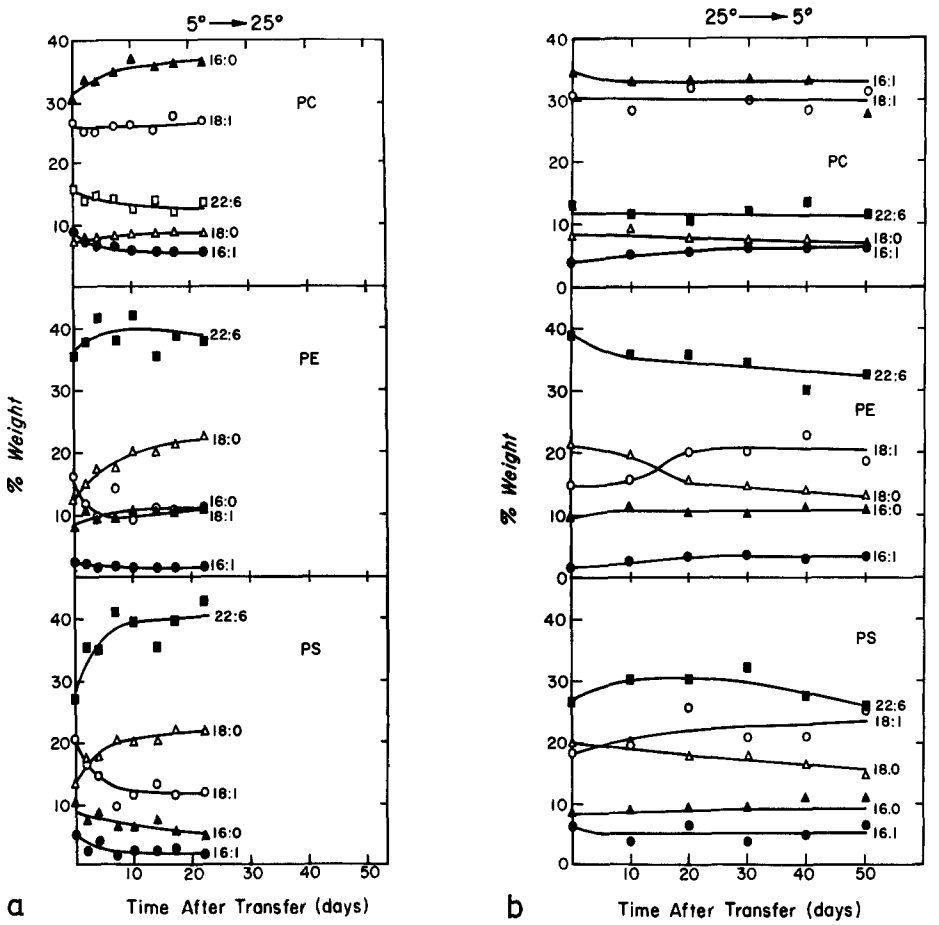

Fig. 3a and b. Time course of changes in the composition of principal fatty acids of three phospholipid fractions, choline phosphoglycerides (*PC*), ethanolamine phosphoglycerides (*PE*) and serine/inositol phosphoglycerides (*PS*). Values expressed as percent of total fatty acid weight in each fraction at specified days after transfer (**a**) from 5 to 25 °C and (**b**) from 25 to 5 °C. Data points represent measurements from pooled synaptosomes of all animals at that time after transfer. Numbers to right of each curve give length of carbon chain and number of double bonds, respectively

decrease in 18:1 while PS showed increased proportions of 18:0 and 22:6 with reduced proportions of 18:1, although for 22:6 this interpretation relies heavily upon the 5 °C steady state acclimated value. Changes in fatty acid composition were generally completed after 10–15 days at 25 °C, although some fatty acid species, notably 16:1 in PE and PC as well as 18:0 in PE and 22:6 in PC had apparently not reached their steady state values.

On transfer of goldfish from 25 °C to 5 °C no changes were evident in PC apart from a small but consistent increase in the proportion of 16:1. In PE there was a marked decrease in 18:0 with a corresponding increase in 18:1, while in PS the proportion of 18:0 decreased and 18:1 increased. The changes in 18:0 and 18:1 of the PE fraction showed a lag of approximately

Table 1. Summary of total percentage of saturated, monounsaturated and polyunsaturated fatty acids in choline phosphoglycerides (PC), ethanolamine phosphoglycerides (PE) and serine/inositol phosphoglycerides (PS) of synaptosomes isolated from the brains of goldfish before transfer and after 22 days at 25 °C and 50 days at 5 °C

Transfer	PC		PE		PS	
	5 °C → 25 °C for 22 days		5 °C → 25 °C for 22 days		5 °C → 25 °C for 22 days	
Saturated	39.80	46.01	27.12	36.45	27.05	27.94
Monounsaturated	35.95	32.78	21.12	12.53	27.26	13.90
Polyunsaturated	24.25	21.21	51.77	51.03	45.72	58.15
Ratio[a]	0.66	0.85	0.37	0.57	0.37	0.39

Transfer	PC		PE		PS	
	25 °C → 5 °C for 50 days		25 °C → 5 °C for 50 days		25 °C → 5 °C for 50 days	
Saturated	44.35	39.38	31.81	27.18	33.51	28.64
Monounsaturated	35.08	39.66	16.41	22.47	26.21	32.42
Polyunsaturated	20.58	20.95	51.78	50.35	40.28	38.95
Ratio[a]	0.80	0.65	0.47	0.37	0.50	0.40

[a] Ratio of saturated to unsaturated fatty acids

10 days followed by a rapid change over the succeeding 10 days. The changes in 18:0 and 18:1 of PS, by contrast, occurred progressively from the start of the experiment and apparently had not been completed after 50 days at 5 °C. Differences in the fatty acid composition compared with analyses reported previously (Cossins, 1977) are probably due to technical differences as well as to seasonal and dietary differences between the stocks of goldfish used.

Table 1 summarizes the observed modifications in the fatty acid composition by comparing the accumulated saturated, monounsaturated and polyunsaturated fatty acids of the three phospholipid fractions for goldfish adapted to 5 °C and 25 °C (i.e., zero time values) with those transferred to 25 °C for 22 days and to 5 °C for 50 days, respectively. The general trends become clear with an overall increase in fatty acid unsaturation during acclimation to 5 °C and a decrease during acclimation to 25 °C. The changes in the proportion of saturated fatty acids were compensated mainly by changes in the proportion of monounsaturated fatty acids, of the Δg series, suggesting that Δg desaturase activity is modulated by temperature. Polyunsaturated fatty acids of the ω3 and ω6 families, by contrast were relatively unaffected by acclimation treatment with the exception of the PS fraction in the 5 °C to 25 °C transfer experiment where there was a paradoxical increase in the proportion of 22:6 after 22 days at 25 °C. This interpretation depends upon the low zero-time value for 22:6, and must be considered in light of the appreciable scatter of data for this fatty acid.

Table 2. Correlation coefficients (r) for (1) the relationship between membrane viscosity (expressed as polarization) and the ratio of saturated to unsaturated fatty acids; and (2) the relationship between membrane viscosity and the unsaturation index (U.I.)

Experiment	*r* values					
	PC		PE		PS	
	Ratio	U.I.	Ratio	U.I.	Ratio	U.I.
25→ 5	0.89[a]	0.08	0.68	0.10	0.52	0.26
5→25	0.86[a]	0.92[a]	0.97[b]	0.33	0.44	0.57

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

[a] $P = < 0.01$

[b] $P = < 0.001$

The ratio of saturated to unsaturated fatty acids (Table 2) for PC obtained after 50 days at 5 °C and 22 days at 25 °C agreed closely with the values obtained for animals acclimated at 5 °C and 25 °C for several months. The agreement for PE and PS was also close for animals held at 5 °C for 50 days and long-time 5 °C acclimated goldfish, but not for the animals held at 25 °C for 22 days or for extended periods. Membrane viscosity (expressed as polarization) correlates more successfully with the molar ratio of saturated to unsaturated fatty acids than with an unsaturation index for both transitions (Table 2). The correlation coefficients are particularly significant for PC, suggesting that alteration in the saturation ratio for this fraction is primarily responsible for membrane viscosity.

The rate of change of fatty acid composition during the 5 to 25 °C transfer was faster (10–15 days to 25 °C acclimated values) than for the transition from 25 to 5 °C (20 days to 5 °C acclimated values). The proportions of 18:0 and 18:1 in PE after transfer from 25 to 5 °C showed a lag of approximately 10 days, after which the proportions changed rapidly over a period of 10 days to the 5 °C acclimated levels.

Discussion

The behavioral responses during exposure to either heat or cold are similar (Friedlander et al., 1976) with marked hyperexcitability, followed by motor incoordination, loss of righting ability and finally, coma. The time course of changes in the critical temperature for these endpoints is faster during transfer of 5 °C acclimated goldfish to 25 °C, than for the opposite transfer and is essentially parallel for the three deficits. By contrast, time-courses for acclimation of several oxidative enzymes (Sidell et al., 1973) are more irregular than behavioral changes. The time for behavioral resistance adaptation at 25 °C is shorter than for changes in cytochrome oxidase and succinic dehydrogenase whereas at 5 °C the times to equilibrium are similar. These differences support other

evidence (Prosser, 1973) that behavioral resistance adaptation is not due to changes in energy metabolism.

Behavioral responses to heat and cold correlate well with temperatures which cause synaptic failure in the central nervous system and not with temperatures which cause cessation of axon conduction (Friedlander et al., 1976). For example, hyperexcitability occurs at temperatures which result in block of inhibitory synapses of the cerebellum. Hyperexcitability on warming as measured by increased swimming occurs at slightly higher temperatures than hyper-reflexia (Fig. 2 in Friedlander et al., 1976). The loss of equilibrium correlates with block of some excitatory synapses and with changes in neuronal discharge patterns, and coma correlates with block of lower brain centers. For synapses of the central nervous system it is difficult to separate presynaptic and postsynaptic events although evidence from neuromuscular junctions suggests that both pre- and postsynaptic functions are affected by extreme temperatures (White, 1976). For synaptic membranes, any functional change induced by acclimation must reflect some change in the chemical composition or structure of the synapse. A first approach to the elucidation of this adaptation is to examine the chemistry of a preparation rich in synaptic elements (synaptosomes). Current concepts of the structure of biological membranes suggest major structural and functional roles for the phospholipid bilayer (Singer and Nicholson, 1972; Singer, 1974). The thermodynamic state or 'fluidity' of this hydrophobic environment has marked effects upon a wide variety of membrane-associated functions and it appears that the maintenance of the appropriate or 'optimal' fluidity is a major factor in the physiology of the membrane (Sinensky, 1974; Esser and Souza, 1974; Cullen et al., 1971). Membrane fluidity of a synaptosomal preparation as measured by the rotational characteristics of an exogenous fluorescence probe showed compensatory changes with temperature acclimation such that 5 °C acclimated goldfish possess synaptosomal membranes with a significantly higher fluidity than 25 °C acclimated goldfish when both were measured at an intermediate temperature (Cossins, 1977). The microviscosity of synaptosomal membranes for both 5 °C and 25 °C acclimated goldfish at their respective heat coma temperatures are almost identical, suggesting that the changes in membrane viscosity that occur during acclimation are of the correct magnitude to account for the changes during acclimation in the critical temperatures which result in behavioral defects. The early time course of changes in synaptosomal membrane fluidity differs from that of behavioral changes but the overall times to attain the new acclimated values are similar. These observations suggest a correlation but not necessarily a direct causal relationship between behavioral parameters and fluidity of synaptosomal membranes. No previous evidence exists for such a relationship.

A precise similarity between the two time courses need not necessarily be expected since the behavioral measurements probably reflect changes in a relatively restricted population of synapses in higher brain centers, while the estimate of membrane fluidity used here provides a weighted average for all membrane types in the preparation. Thus changes in membrane viscosity of only a small proportion of synaptosomes, of particular membranes within synaptosomes or in specific microenvironments within a particular membrane, may occur with

no measurable effect upon bulk membrane fluidity as measured by the fluorescence polarization technique. Electron microscopic examination of the synaptosomal preparation has indicated that myelin contaminants were absent and that approximately 5% of membranous structures were distinctly different from the synaptosomal population (Cossins, 1977) and their contribution to the results is probably correspondingly small. However, synaptosomes are typically composed of several distinct membrane types such as pre- and postsynaptic membranes, associated neurilemma, intrasynaptic mitochondria and synaptic vesicles, each of which may respond differently during thermal acclimation. For example, mitochondria of green sunfish liver exhibit a marked homeoviscous response whereas the sarcoplasmic reticulum of goldfish muscle shows none (Cossins, Christiansen and Kent, in preparation). The partial compensation of synaptosomal membranes (Cossins, 1977) may be the weighted average of fully, partially and non-adapting membranes. Clearly, the homeoviscous response is not a ubiquitous property of all biological membranes and further work is required to demonstrate which membranes of the synaptosome are affected during thermal acclimation.

Previous studies have shown in both artificial and biological membrane systems that the molecular mobility of membrane constituents is increased by an increased proportion of unsaturated constituent hydrocarbon chains, principally through the steric disruption of London-Van der Waals forces between adjacent hydrocarbon chains by the kinked cis-olefinic bond (Salem, 1962; Demel et al., 1967). Analyses of several fish species have repeatedly demonstrated that the membrane phospholipids of cold-acclimated fish are more unsaturated than those of warm-acclimated fish (for review see Hazel and Prosser, 1974), although the precise details vary according to type of animal and preparation examined. Membrane phospholipids of a thermotolerant strain of *Tetrahymena* are more unsaturated when the cells are grown at 15° than at 39.5 °C (Fukushima et al., 1976). In the present experiments the simultaneous and opposite changes in the proportion of 18:0 and 18:1 of the PE and PS fractions during cold and warm acclimation appear to be the principal modification of membrane fatty acid composition. Changes in the proportion of other fatty acids are less marked but are in the same general direction, so that at each fully-acclimated state there is a clear correlation between the proportion of unsaturated fatty acids of membrane phospholipids and synaptosomal fluidity, as reported previously (Cossins, 1977). The lag of approximately 10 days in changes in the proportion of 18:0 and 18:1 of PE during the transfer from 25 °C to 5 °C suggests that sudden exposure to cold impairs the acclimatory ability of the synthetic machinery. This is reflected in the 20 day lag of changes in synaptosomal fluidity, but not in the behavioral studies where critical temperatures for the various behavioral endpoints showed an immediate and progressive transition to the 5 °C acclimated state. As before, this discrepancy may be reconciled by assuming that other properties besides viscosity are critical for synaptic function, and that changes in behavioral parameters during acclimation may be due to a restricted population of synapses which exhibit simultaneous changes in fluidity and fatty acid composition while the majority of synapses exhibit slightly different transitional kinetics. The membrane viscosity may be influenced

by protein components as well as by phospholipids, and specific membrane proteins may change in concentration and type during temperature acclimation. Both quantitative and qualitative changes during acclimation have been demonstrated for such specific enzymatic proteins as acetylcholinesterase (Baldwin and Hochachka, 1970; Prosser, 1975 for references).

Changes in both behavioral endpoints and synaptosomal viscosity are extremely complex and the unsaturation of fatty acids is probably only one of many contributing factors. However, all three kinds of measurements are correlated in time and direction of change. Indeed, the differences in temperature of block of certain behavioral responses between 5 °C- and 25 °C-acclimated goldfish (5.5 °C) agree closely with the differences in the steady state values of the membrane viscosity for fish acclimated to the two temperatures (approximately 6 °C). It is concluded that changes in the critical temperatures for behavioral effects during several weeks of acclimation to either high or low temperatures are closely related to the changes in fluidity of synaptic membranes and these are related to unsaturation of their constituent phospholipids.

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