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MINI-REVIEW

The Adaptation of Biological Membranes to Temperature and Pressure: Fish from the Deep and Cold

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Abstract

The homoeostatic regulation of bilayer order is a property of functional importance. Arguably, it is best studied in those organisms which experience and must overcome disturbances in bilayer order which may be imposed by variations in temperature of hydrostatic pressure. This article reviews our recent work on the adaptations of order in brain membranes of those fish which acclimate to seasonal changes in temperature or which have evolved in extreme thermal or abyssal habitats. The effects of temperature and pressure upon hydrocarbon order and phase state are reviewed to indicate the magnitude of the disturbances experienced by animals in their environments over the seasonal or evolutionary timescale. Acclimation of fish to altered temperature leads to a partial correction of order, while comparison of fish from extreme cold environments with those from temperate or tropical waters reveals a more complete adaptation. Fish from the deep sea also display adaptations of bilayer order which largely overcome the ordering effects of pressure.

Key Words: Membrane fluidity; fish membranes; homeoviscous adaptation; temperature; hydrostatic pressure; acclimation.

Introduction

Recent concepts of membrane structure emphasize the functional significance of molecular mobility within the phospholipid bilayer (Quinn, 1981;

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Shinitzky, 1984; Lenaz and Castelli, 1985). Under physiological conditions, the bilayer is generally thought to be in a disordered or fluid condition (Seelig and Seelig, 1980), the degree of molecular mobility and structural order of the hydrocarbon chains having a direct effect upon the dynamic structure, segmental mobility, and catalytic activity of at least some membrane-bound enzymes. It is also generally accepted, if only implicitly, that the fluidity of the bilayer is precisely regulated. This is mainly because the chemical composition and measured order of any particular cellular membrane are quite consistent. However, the most direct method of investigating the regulation of membrane order is to disturb the system and to observe corrective responses. Temperature and hydrostatic pressure are the most powerful disturbing influences, and studying the adjustment of membranes to these modalities reveals, in a particularly dramatic way, the capacity of cells, in general, to regulate adaptively the structure and functional properties of their constituent membranes.

Perhaps the most consistently observed adaptive responses of microorganisms, plants and animals to temperature variations involves changes to the lipid composition and dynamic structure of their cellular membranes (Cossins, 1983; Hazel, 1984). Invariably, cold adaptation is associated with the incorporation of greater proportions of unsaturated fatty acids in membrane lipids and this causes, by means that will be discussed later, an increase in the disorder of the bilayer that overcomes the direct ordering influences of cold. This homeostatic response has been termed "homeoviscous adaptation" (Sinensky, 1974). Although originally applied to temperature adaptations in bacteria (Sinensky, 1974), protozoans (Thompson, 1980), and fish (Cossins, 1977), the concept of homeoviscous adaptation has been applied to other agents that perturb membrane structure. Indeed, compensatory behavior has been described in response to high hydrostatic pressure (Macdonald and Cossins, 1985), to membrane-fluidizing anesthetics (Littleton, 1983), and to dietary alterations in membrane lipid composition (Cossins and Sinensky, 1984).

Fish are particularly useful experimental organisms for studies of molecular adaptation. They have succesfully invaded virtually every aquatic habitat from the polar oceans at $-2°C$, to tropical ponds at 40°C, and to the deep oceans where pressures of up to 1000 atm compound the effects of extreme cold (2–3°C). (Pressure increases by 10 MPa or 100 atmospheres for each kilometer of depth.) In addition, many species experience daily or seasonal variations in temperature and have developed a rather versatile physiology suited to these varying conditions, a phenomenon termed "acclimatization." The deleterious effects of such environmental variations are felt in these animals at the cellular and molecular levels of organization, and this necessitates a range of compensatory adaptations to permit normal cellular

function; responses that are not observed, for example, in the mammals with their constant body temperature (Cossins and Bowler, 1987).

In this review, we describe our recent work on the compensatory responses of fish membranes to environmental perturbation. We discuss first the direct effects of temperature and hydrostatic pressure upon membrane structure because it is only through an understanding of these effects that compensatory responses can be recognized. We then describe studies that demonstrate adaptive responses to temperature and pressure, respectively. Finally, we consider what these studies tell us about the general principles that are important in the design and construction of membranes.

The Problem: Effects of Temperature and Pressure on Bilayer Order

Although membrane phospholipids are anisotropically arranged in ordered semicrystalline arrays, there may be, nevertheless, a significant amount of molecular motion and disorder, especially in the liquid-crystalline state (Quinn, 1981). This motion may be of several distinct types, ranging from the flexing of hydrocarbon chains by rotation of carbon–carbon bonds to the wobbling motion of entire molecules, to the lateral displacement of molecules. The resulting condition is commonly described as being fluid, though it must be recognized that, because of the orderly arrangement of phospholipids in bilayers, the fluidity of this compartment is quite distinct from the bulk fluidity of hydrocarbon solvents, such as paraffin (van der Meer, 1984). In principal, a detailed understanding of "fluidity" can be gained by analyzing the movement of atoms or segments of hydrocarbon chains using nuclear magnetic resonance (NMR) spectroscopy, and it is now quite clear from NMR studies that treating the bilayer interior as a bulk compartment is a gross oversimplification. Nevertheless, much useful information relating to the physiologically interesting aspects of membrane dynamic structure can be gained by the use of spectroscopic probes.

Of the spectroscopic techniques available to study membrane structure, fluorescence polarization spectroscopy has proved to be the most popular (Shinitzky and Barenholz, 1978). This is mainly because it is a relatively simple technique requiring only a straightforward modification to inexpensive laboratory spectrofluorimeters. The instrumentation is to some extent portable and this has allowed us to perform measurements on fresh material on board ship at sea. Moreover, the technique is exquisitly sensitive, so that low probe/phospholipid ratios are achievable and only small quantities of biological material are required. Both electron-spin resonance and fluorescence polarization probes tend to average out the microscopic details that may be provided by NMR spectroscopy and, depending upon the

structure and binding characteristics of the probe, may emphasize one or another aspect of the fluid condition.

The most commonly used probe is 1,6-diphenyl-1,3,5-hexatriene (DPH). This partitions into the hydrophobic domains where its fluorescence intensity is enhanced $\sim 10^4$-fold (Shinitzky and Barenholz, 1978). It appears that DPH distributes itself between at least two sites, one parallel to the hydrocarbon chains and the other between the two bilayers (Mulders *et al.*, 1986); the resulting fluorescence anisotropy is thus a weighted average of these different binding sites. Fluorescence anisotropy determined under continuous illumination (i.e., "steady state" anisotropy, r_s) indicates the extent to which an oriented population of fluorophores becomes disordered during the fluorescence lifetime. Early workers considered anisotropy to be a measure of the effective viscosity of the immediate microenvironment of the fluorophore [i.e., "microviscosity" (Shinitzky and Yuli, 1982)] though this has been superseded by a more complex treatment that includes both a static ordering term, r_{inf}, which is related to the order parameter, and a dynamic term, the rotational diffusion coefficient (Dale *et al.*, 1977; Kinosita *et al.*, 1977; Heyn, 1979; Jahnig, 1979). Recently, time-resolved anisotropy decays have been used to determine the fourth-rank orientational order parameter (van der Meer *et al.*, 1984). The contribution to depolarization from the dynamical motion of the rigid probe is small in ordered membranes, but progressively increases as the bilayer becomes less disordered. Thus, for most biological membranes, which are relatively ordered, the steady-state anisotropy is closely related to the ordering term and provides a convenient measure of membrane order as experienced by the fluorophore (van Blitterswijk *et al.*, 1981). Order parameters calculated from anisotropy measurements give reasonably good agreement with values of segmental order parameter obtained at carbon-10 to carbon-12 segments from deuterium NMR spectroscopy (Heyn, 1979; Jahnig, 1979). Steady-state anisotropy ranges from 0.3 to 0.34 in fully ordered gel state (e.g., dipalmitoyllecithin bilayers $< 40°C$) to < 0.1 in disordered bilayers [e.g., egg lecithin at room temperature (Shinitzky and Barenholz, 1978)].

It is worth pointing out that uncertainties regarding the positional distribution of DPH or the precise interpretation of anisotropy in terms of fluidity do not prevent the interesting comparison of different membranes. Studies of membrane adaptation are, by and large, comparisons of membranes from one species with membranes from another, or between membranes of differently treated individuals of the same species. Provided the membranes are sufficiently similar in terms of lipid composition, ultra- structure, function, probe-binding sites etc., then differences in DPH anisotropy will indicate *differences* in the order of fluidity of the respective membranes.

Some of the uncertainty due to the ill-defined positional distribution of DPH may be resolved by the use of "tethered" probes such as trimethylammonium-DPH (TMA-DPH) and β-parinaric acid. Comparative experiments using these probes has produced essentially the same results as those obtained with DPH (M. K. Behan, unpublished observations).

Temperature

An increase in temperature has two separate, but related, effects upon bilayer structure. First, it leads to a progressive decrease in DPH anisotropy. Plots of log anisotropy (or derived functions such as microviscosity) as a function of temperature or reciprocal temperature are frequently linear especially for phospholipid liposomes in the liquid-crystalline state (Shinitzky, 1984). The temperature dependence of DPH anisotropy is influenced by the chemical composition of the bilayer lipids; thus, cholesterol reduces the Arrhenius activation energy of artificial bilayers (Shinitzky and Inbar, 1976).

Second, warming may alter the physical state of phospholipids from a gel to the liquid-crystalline state or may alter the proportion of the two if they coexist (Melchior and Steim, 1976; Lee, 1983). Nonlinear Arrhenius plots may be induced by phase transitions in liposomes composed of well-defined phospholipids, though whether nonlinear plots necessarily indicate a phase transition in biological membranes composed of complex mixtures of lipids and proteins is controversial. The evidence for phase transitions is controversial. The evidence for phase transitions in eukaryotic membranes over the physiological range of temperatures or pressures is frequently not persuasive and, even in instances where transitions have been observed by calorimetric techniques, they generally include only small ($< 5\%$) proportions of the bilayer lipids (McMurchie *et al.*, 1983). The functional significance of phase transitions or of phase separations is still not well defined in eukaryotic membranes, though, in the mycoplasma *Acholeplasma*, the phase state of the membrane lipids influences several important membrane functions (McElhaney, 1984). However, because transitions are so dramatic, it must be recognized that they pose severe problems for organisms whose membranes may normally exist in a liquid-crystalline conditions. Indeed, the existence of phase transitions has long been implicated as the principal reason why chill-sensitive plants cannot withstand freezing or why mammals cannot withstand body temperatures below $\sim 20°C$ (Raison *et al.*, 1988). The regulation of phase state or the degree of phase separation may well be of greater importance than the fine-tuning of membrane order. DPH anisotropy is not the best technique for determining the extent of transitions, and the adaptive regulation of phase state in eukaryotes, at least, is an important area for future study.

Hydrostatic Pressure

Hydrostatic pressure compresses the bilayer anisotropically, the lateral intermolecular spaces compressing markedly while the acyl chains straighten and slightly extend the thickness of the bilayer (Braganza and Worcester, 1986). The bulk compressibility of dipalmitoylphosphatidylcholine bilayers is $\sim 1\%$ MPa^{-1} (Liu and Kay, 1977). These dimensional changes are less important in the present context than the accompanying changes in molecular order and motion that pressures in the 1–100 MPa range produce. These have been reviewed recently by Macdonald (1987) and by Wong *et al.* (1988). Again, a convenient measure of the perturbation caused by pressure is provided by fluorescence spectroscopy. DPH anisotropy typically increases 0.0034 per 10 MPa^{-1} (= 1000 m depth) in fish brain membranes (Chong and Cossins, 1983). This is, perhaps, more easily understood by reference to its temperature equivalent (i.e., the increase in temperature required to offset the ordering effects of an increase in pressure). In pure phospholipid liposomes, this is 0.17–0.29°C MPa^{-1} while, for the more complex natural membranes, it is 0.13–0.21°C MPa^{-1} (Macdonald, 1987).

Measurements such as those based on DPH assume that pressure causes no change in the partitioning of the probe between the anisotropic region of the bilayer and the relatively disordered interior region. Other fluorescent probes such as PRODAN do shift their intrabilayer distribution under pressure (Chong, 1988), though there is some evidence that this is not the case with DPH (Chong and Weber, 1983).

Lateral diffusion by small molecules in the plane of the bilayer is reduced by high pressure, consistent with the lateral compressibility mentioned earlier. Excimer formation of 1′-pyrenedodecanoic acid is inhibited, and the calculations based on reasonable (but not unassailable) assumptions show that 100 MPa halves the diffusion coefficient of the probe in erythrocyte membrane at 37°C (Eisinger and Scarlatta, 1987). Excimer formation in unconjugated pyrene is also inhibited by pressure, but the interpretation of this probe's behavior is somewhat contentious. Instead of measuring lateral diffusion, it may report excimer formation in microaggregates in relatively polar regions of the bilayer (Macdonald *et al.*, 1988).

Hydrostatic pressure may also induce isothermal phase transitions and a variety of studies have shown that the transition temperature increases linearly with pressure in accordance with the Clausius–Clapyron relationship $\partial T/\partial P = T \cdot \Delta V/\Delta H$ in which T is the transition temperature, P is the pressure, ΔV is the molar volume change of the transition, and ΔH is the enthalpy change of the transition. Typical values of $\partial T/\partial P$ are 0.2–0.3°C MPa^{-1} These values may be taken to indicate that transitions are rather insensitive to the pressure variations normally encountered by animals

undertaking vertical migrations. However, provided that the transition is cooperative and that pressure is applied at or close to the transition temperature, then small pressure variations may have very significant effects. Pressure-induced phase transitions may influence the functional properties of membrane-bound enzymes (De Smedt *et al.*, 1979).

The problems posed by life in the deep oceans may be appreciated by adding the ordering effect of high hydrostatic pressures to the ordering induced by the cold in those environments. Thus, 100 MPa (1000 atm) is equivalent to a cooling of 13–21°C below the ambient temperature of 2°C, to give −11 to −19°C. Thus, the membranes of animals living at these extreme depths must be functional at conditions that resemble a domestic deep freeze. Interestingly, this low temperature is similar to the lowest at which life (growth or movement) has been observed at atmospheric pressures (Vallentyne, 1963).

High pressures may coexist with high temperatures in oil-well and other subterranean fluids, which are colonized by bacteria, and in hot vents in the ocean floor. The latter occur at depths of 2000–4000 m (20–40 MPa) and sustain a local bacteria flora on which invertebrates and fish feed (Jannasch, 1985; Grassle, 1985). The latter exist in steep thermal gradients between the undiluted vent fluid (> 100°C) and the normal water at 2°C. These higher-than-normal temperatures oppose the ordering influence of pressure, and bilayer adaptation may differ from that in the majority of deep-sea organisms.

The Potential for Adaptive Responses

In overcoming the direct effects of temperature or pressure upon lipid bilayers, two extreme forms of adjustment may be recognized (Fig. 1). The first, which is conventionally termed a "translation," involves a change in the fluidity of the membrane without any change in its temperature dependence or pressure dependence. This change offsets to a greater or lesser extent the perturbation suffered by the membrane and involves a translation of the curve relating fluidity to temperature or pressure along the abcissa. The second response involves a change in the temperature coefficient or pressure coefficient of fluidity so that the curves are rotated with respect to each other. Identifying the nature of the adaptive response may provide information on the underlying mechanism since certain compositional alterations (e.g., fatty acid saturation) may produce a translation while others (e.g., cholesterol) may produce a rotation. Given the diversity of lipids present in biological membranes and the dramatic differences in thermotropic behavior of phospholipids containing saturated, mono-, or polyunsaturated fatty acids, there

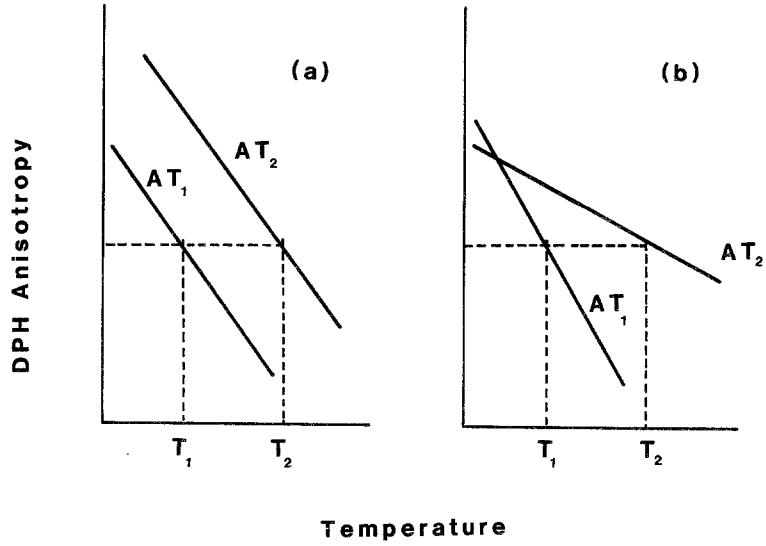

Fig. 1. A schematic diagram to illustrate the principal types of compensatory resonse that occur during thermal acclimation to temperatures T_1 and T_2: (a) A "translational" shift of the curve relating DPH anisotropy with temperature. (b) A change in the slope of the curves to give a "rotational" response. In each case, the difference between the curves for each acclimation temperature is such that identical values of anisotropy occur at each acclimation temperature. A similar relationship holds for compensatory responses to hydrostatic pressure.

is plenty of scope for regulatory adjustment of fluidity leading potentially to a degree of independence of bilayer order from these potent thermodynamic factors.

It is also necessary to define two time scales over which adaptive responses may occur. The first occurs during the lifetime of an individual as it responds adaptively to altered conditions or demands. It may be recognized by the comparison of identical animals that have different thermal or pressure experiences, a process termed "acclimation" for laboratory-controlled treatment or "acclimatization" for naturally occurring seasonal exposure (Cossins and Bowler, 1987). The second timescale occurs during the evolutionary development of a population or species to an altered environment and may be recognized by the interspecific comparisons of animals of known thermal history. In that animals which experience and acclimatize to seasonal or diurnal variations must maintain a flexible response, there is reason to believe that their adjustments may be less profound than those that may occur during evolutionary development of species to particular thermodynamic niches.

Adaptations to Temperature

Acclimations

The effects of thermal acclimation upon DPH anisotropy of a large number of different membrane fractions have been examined. Without exception, where differences were observed between the membranes of cold- and warm-acclimated fish, the responses were simple translations with no obvious rotation. Given this generalization, the degree to which the acclimation response offsets the direct effects of the temperature shift may be expressed as the ratio of the shift of the anisotropy–temperature curves along the temperature axis to the difference in acclimation temperatures, a parameter termed "homeoviscous efficacy" (HE). A value of 1 indicates identical DPH anisotropies for membranes at their respective acclimation temperatures while a value of 0 indicates no adaptive response.

Table I lists the HE's determined so far. Most membranes displayed values of HE between 0.2 and 0.5, and none showed complete compensation (HE = 1). The most complete data set is for the brain membrane fractions of goldfish where HE increases in the order myelin > synaptic > mitochondrial. There are other examples where different subcellular fractions from the same tissue and probably the same cell type exhibited different HE's, with the more metabolically active membranes showing the highest values. Whether this is due to a different responsiveness *per se* or to different rates of turnover of membrane components in the different fractions is not clear. Two membrane fractions showed no compensation, namely, the

Table I. A Comparison of Homeoviscous Efficacy (HE) inFish Membranes

Species	Membrane fraction	Acclimation temp (°C)	HE	Reference
Goldfish	Synaptosomal	5–25	0.3	Cossins (1977)
	Brain myelin	5–25	0.21	Cossins and Prosser (1982)
	Brain synaptic	5–25	0.36	Cossins and Prosser (1982)
	Brain mitochondria	5–25	0.44	Cossins and Prosser (1982)
	Sarcoplasmic reticulum	5–25	0.0	Cossins *et al.*, (1980)
Lepomis cyanellus	Liver microsomes	5–25	0.3	Cossins *et al.* (1980)
	Liver mitochondria	5–25	0.5	Cossins *et al.* (1980)
Carp	Intensinal mucosa,			
	Brush border	8–30	0.0	Lee, J. A. C.[a]
	Basolateral	8–30	0.75	Lee, J. A. C[a]
Trout	Erythrocytes	3–21	0.2	Raynard, R. B.[a]
	Brain synaptic	3–21	0.3	Behan, M. K.[a]

[a] Unpublished observations.

sacroplasmic reticulum of goldfish and the brush border of carp intestinal mucosa. A particularly good example of differential responses from different fractions from the same tissue is in the intestinal mucosa of temperature-acclimated carp. The basolateral fraction showed the greatest HE of all membranes examined so far (0.75) while the brush-border fraction showed no difference.

Interspecific Comparisons

The comparison of membranes from different species requires the broad similarity of membranes from the various species in respect of the

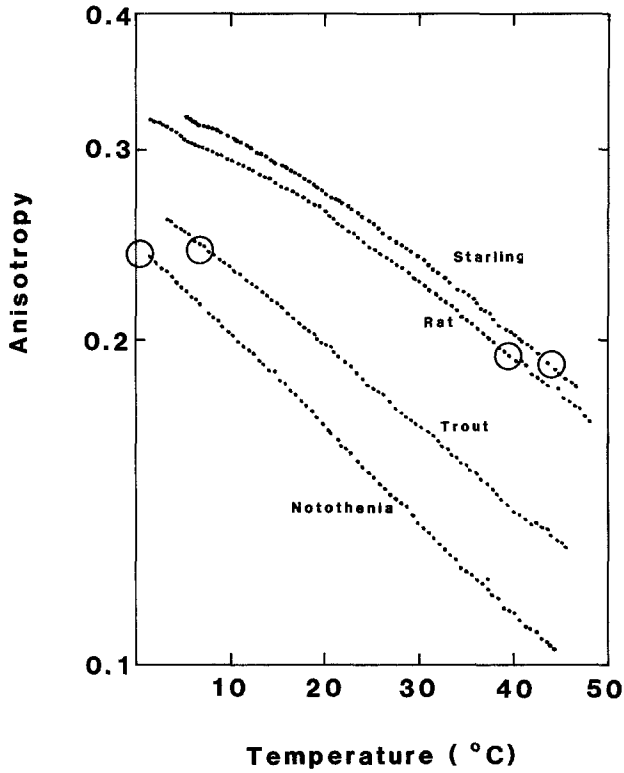

Fig. 2. Evolutionary homeoviscous adaptation for brain membranes of two fish species, a bird, and a mammal. *Notothenia neglecta* were obtained from the Antarctic Ocean by the British Antarctic Survey. Trout were acclimated to 6°C. The values of anisotropy at the respective environmental (fish) or body temperature (bird, mammal) have been indicated with a large open circle. Note the anisotropies have been plotted on a log scale. The interspecific differences are sufficiently large and in the appropriate direction to provide similar anisotropies at their respective body temperatures (A. R. Cossins, M. K. Behan, G. Jones, and K. Bowler, unpublished observations).

necessarily impure membrane fractions. Arguably this is best satisfied in the brain, where neuronal structure and organization at the cellular level is relatively constant, certainly within the teleosts and probably within the vertebrates as a whole. Figure 2 compares the DPH anisotropies for brain synaptic fractions from fish species from different thermal environments together with corresponding fractions from a small mammal and a bird. There are clear-cut interspecific differences that correlate with the thermal experience of the species; thus, anisotropy increases in the order *Notothenia* (from the Antarctic Ocean, 0°C), trout (acclimated at 6°C), rat (body temperature, 37°C), and starling (42°C). Again the curves were broadly parallel, indicating a translational response. Moreover, the interspecific differences were sufficiently large to provide roughly similar values of anisotropy at their respective body temperatures. Thus, DPH anisotropy for rat at 37°C was similar to the corresponding value for the Antarctic fish species *Notothenia* at 0°C, suggesting that HE for this interspecific comparison approaches unity.

Adaptations to Pressure

We have seen that, while high pressure does not precisely mimic low temperature in its effects upon membrane bilayers, both thermodynamic parameters influence membrane order. The theory of homeoviscous adaptation predicts that adaptive changes to high pressures should be similar to those of low temperature. The major disordering mechanism used by fish during cold adaptation, the insertion of *cis*-double bonds into membrane fatty acids (Hazel, 1984), appears well suited to pressure compensation. The steric consequences of introducing the 30° kink of a *cis*-double bond into a saturated chain are obvious (Brener, 1984; Bell *et al.*, 1986); a large free volume is created and intermolecular spaces are increased to permit greater conformational freedom of the hydrocarbon chains. The role of polyunsaturated fatty acids in membrane structure is less clear, and it is likely that fatty acids with 4, 5, or 6 double bonds may have some unusual and functionally important packing characteristics (Stubbs and Smith, 1984; Applegate and Glomset, 1986).

Two criteria have been adopted to determine the role of homeoviscous adaptation in deep-sea fish. One was the direct comparison of DPH anisotropy of membranes from deep-sea and shallow-water fish species, and the second was a comparison of the fatty acid composition of these same membranes. Although the first measurements of bilayer fluidity of membranes from deep-sea organisms were carried out on deep-sea fish (Cossins and Macdonald, 1982), microorganisms have now been more

extensively studied (DeLong and Yayanos, 1985; Wirsen *et al.*, 1987). In the former case, natural populations have been used in preference to experimentally acclimated preparations. The technical problems of maintaining fish in high-pressure aquaria are considerable. In any case, relatively large pressure excursions would be required to elicit much change in DPH anisotropy while it is known that lesser pressure changes may exert adverse effects upon excitable membranes (Sebert *et al.*, 1986, Macdonald *et al.*, 1987).

We have, therefore, adopted a more practical approach of using membrane fractions of freshly trawled fish from different depths. A number of deep-sea fish species are well known to be confined to the ocean floor and may be reliably trawled in quantity. This requires a specialized research vessel equipped with a winch capable of paying out 12 km of warp to trawl the ocean floor at up to 4 km depth. The most serious technical limitation, however, lies in the preparation of good-quality membrane fractions. High-speed centrifugation is limited at sea by motion of the ship, but we found that an IEC Centra RS refrigerated centrifuge was capable of subjecting 40 ml to 15,000 rpm with the ship under way in a moderate Atlantic swell. Liver mitochondrial, brain synaptic, and brain myelin fractions were prepared using modifications of normal laboratory procedures. DPH anisotropy was immediately determined at 4 ± 0.1°C and atmospheric pressure on a "T"-format polarization fluorimeter specially adapted to smooth out electronically the considerable effects of ship motion upon photomultiplier performance (Cossins and Macdonald, 1984). Fatty acid analyses were performed on frozen material in the conventional manner in our laboratories.

Figure 3 shows the relationship between DPH anisotropy (at 4°C, atmospheric pressure) and depth for brain myelin preparations from several different species that live over different depth ranges. Perhaps the closest interspecific comparison is between two species of the genus *Coryphenoides*, one that inhabits the depth range 400–2000 m (*C. rupestris*) and the other from 2200 to at least 4800 m (*C. armatus*). DPH anisotropy was distinctly lower for *C. armatus*, indicating a lower order in the deep-sea species. Of the three membrane fractions examined, only brain myelin fraction showed a statistically significant regression of DPH anisotropy with the depth of capture (Cossins and Macdonald, 1984), anisotropy decreasing 0.0027 for each 1000 m. This is not dissimilar to the ordering effects of pressure at 0.0034 for each 1000 m, so, assuming that all species have identical pressure coefficients for anisotropy, it appears that interspecific differences determined under standard conditions may well be sufficient to overcome the direct effect of pressure.

Recently, the pressure dependence of membrane order of myelin from deep-sea species has been determined using a high-pressure cuvette chamber similar to that described by Paladini (1980) and some preliminary results are

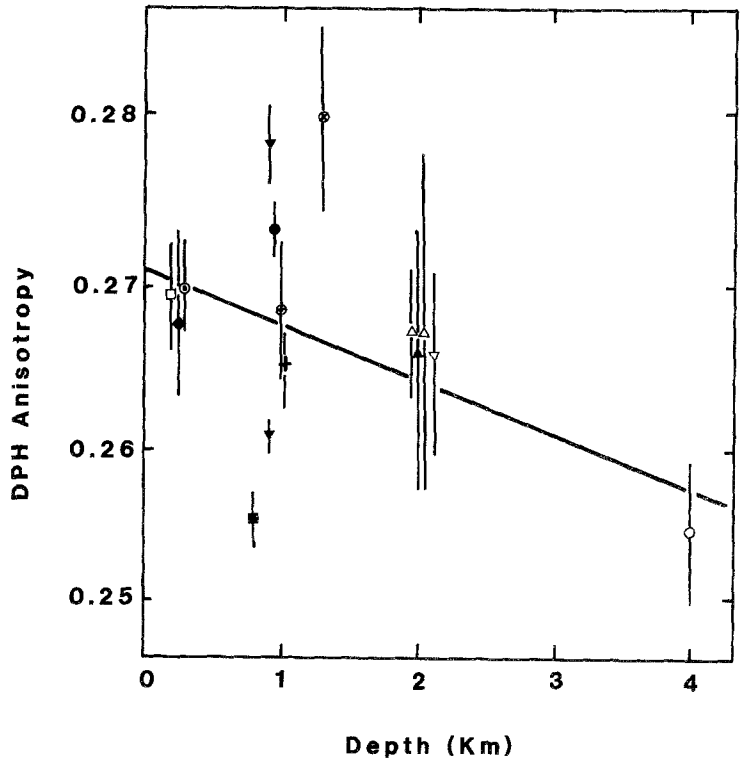

Fig. 3. The relationship between DPH anisotropy and depth of capture for brain myelin membrane fraction of deep-sea and shallow-water fish species. Symbols and error bars represent the mean ± SEM for n individual. The thick line represents the regression with slope of -0.003 and intercept of 0.271, $n = 82, p < 0.001$. Despite the significance of the regression, considerable variation within and between species was not explained by the regression: (○) *Coryphenoides armatus* ($n = 15$); (●) *Nezumia aequalis* ($n = 5$), (△) *Antimora rostrata* ($n = 4$ and $n = 4$)), (▲) *C. rupestris* ($n = 6$), (□) *Lepidorhombus whiffiagonis* ($n = 7$) *Conocara murrayi* ($n = 4$), (■) *Phycis blennoides* ($n = 4$), (▼) *Lepidion eques* ($n = 32$ and $n = 7$), (◆) *Lophius budegassa* ($n = 3$), (+) *Alepocephalus bairdii* ($n = 6$), (⊙) *Helicolenus dactylopetrus* ($n = 4$), (⊕) *Mora moro* ($n = 3$) and (⊗) *Hoplostethus atlanticus* ($n = 4$). Data of Cossins and Macdonald (1984).

shown in Fig. 4. The curves showed a progressive increase of steady-state anisotropy with increasing pressure. The most interesting feature of the results is the broad similarity in pressure dependence of DPH anisotropy in the three species over the biologically relevant linear range of pressures, indicative of translation-type differences. The large open circles indicate the values that occur at 4°C, but under their respective environmental pressures. The interspecific differences are in the appropriate direction and are of the correct magnitude to produce similar DPH anisotropies at their respective environmental conditions.

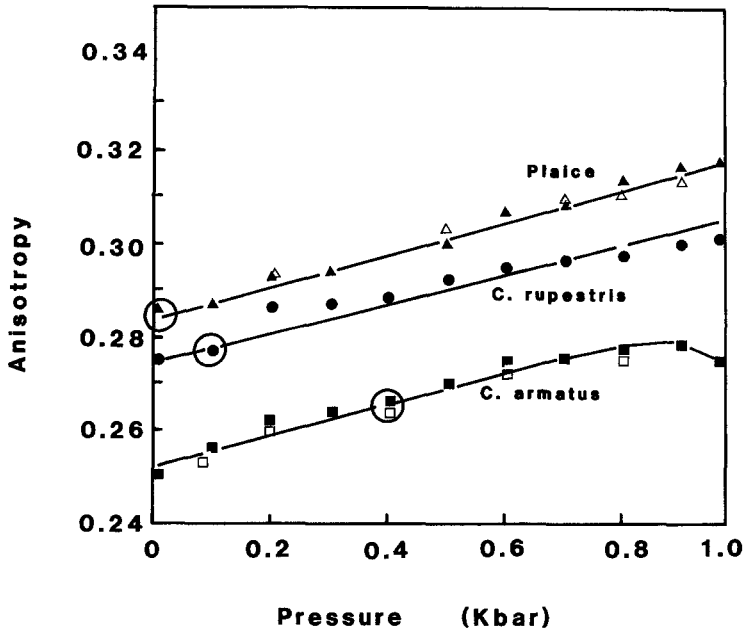

Fig. 4. The pressure dependence of DPH anisotropy at 4°C in brain myelin membrane fraction of the deep-sea species *Coryphenoides armatus* (trawled at 4000 m) and *C. rupestris* (900 m), and the shallow-water plaice *Pleuronectes platessa* (< 100 m). The filled symbols represent values obtained during compression, while unfilled symbols were obtained during decompression. The anisotropy at the hydrostatic pressures and the normal environmental pressure of each species at 4°C is indicated with a large unfilled circle. (M. K. Behan, G. Jones, A. G. Macdonald, and A. R. Cossins, unpublished observations).

Of the membrane fractions isolated at sea, only liver mitochondria were available in sufficient quantity to allow biochemical characterization. The fatty acid composition of the principal phospholipid classes, phosphatidylcholine and phosphatidylethanolamine, for over 40 individuals of 12 species was determained (Cossins and Macdonald, 1986). In both phospholipid classes, the ratio of saturated to unsaturated fatty acids showed a statistically significant regression with depth of capture. Figure 5 shows the relationship for phosphatidylethanolamine, from which it is clear that depth explains much ($\sim 60\%$), but not all, of the interspecific or interindividual variation. Thus, *Lepidorhombus whiffiagonis* had low saturation ratios compared to *Lophius budegassa* even though both were caught at 200 m, and it appears that other factors, such as reproductive and nutritive status, are not without importance. Acceptance for the moment that membrane order is heavily influenced by lipid saturation, this result suggests that liver mitochondria also exhibit homeoviscous adaptation. The lack of correlation of DPH anisotropy

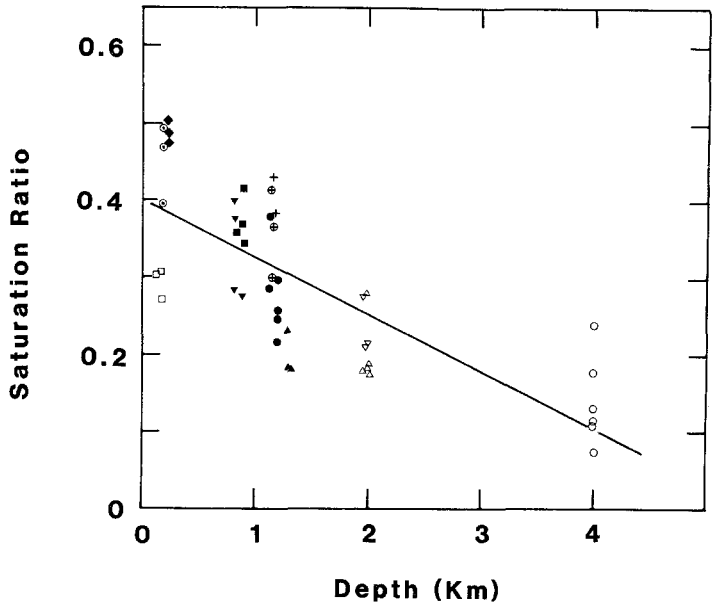

Fig. 5. The relationship between lipid composition for phosphatidylethanolamine of liver mitochondrial membranes and depth of capture. The saturation of fatty acids is represented by the saturation ratio, which is the ratio of saturated to unsaturated fatty acids. Each symbol represents a separate species as described in the legend to Fig. 3. From Cossins and Macdonald (1986).

with depth was probably due to the large interindividual variation in condition, color, and size of the liver and the resulting mitochondrial fractions (Cossins and Macdonald, 1986).

Conclusions

Without exception, the adaptive responses to temperature and hydrostatic pressure described here were translations. The acclimation responses displayed HE's generally in the range 0.2–0.5 and no ideal compensations have been observed. The differences in acclimation temperature lie well within the range for which phospholipids with varying unsaturation can provide equivalent fluidities and it is puzzling why thermal acclimation does not take advantage of this diverse collection of lipids to achieve a more complete compensation of membrane fluidity. In other words, it appears that the constraints that restrict the adaptive responses during thermal acclimation of fish and not biophysical, but biological.

The advantages that follow from any particular adaptive response must be viewed in relation to the costs, metabolic and otherwise, associated with the adaptation. In the case of thermal acclimation, it seems either that the costs of ideal compensation are too high in relation to the benefits or, put another way, that the benefits of ideal compensation are not sufficient to warrant greater responses. The benefits of homeoviscous adaptation follow from the relative independence from seasonal variations of temperature of the many membrane functions that are influenced by bilayer order and the manner in which these contribute to the preservation of whole animal performance despite changes in temperature and pressure. The unacceptable cost may be simply energetic, being related, perhaps, to the metabolic cost of maintaining a sufficiently flexible response. For example, deep-sea fish appear to have rather low metabolic rates as an energetic adaptation to a food-scarce environment (Somero *et al.*, 1983) and this may influence the extent of homeoviscous adaptation in a tissue- and membrane-specific manner. Physiological compensations to temperature occur over the middle range of temperatures, and exposure to lower temperatures may induce noncompensatory energy-saving responses, such as torpor (Cossins and Bowler, 1987).

Interestingly, the greatest acclimation response observed so far in fish is in the basolateral membrane fraction of carp intestinal mucosa, a tissue where compensation of epithelial ion transport involves compensations in basolateral Na^+ pump activity (Gibson *et al.*, 1985). The $(Na^+ + K^+)$-stimulated ATPase is commonly believed to be heavily influenced by bilayer order (see, for example, Sinensky *et al.*, 1979). Large responses have also been found in mitochondrial membranes, which are known to be an important part of the overall cellular response to altered temperature (Wodtke, 1981). By contrast, the two membrane systems that showed no compensation, namely, the sarcoplasmic reticulum of muscle and the brush border of intestinal mucosa, are situations where functional adaptations of the membrane systems may play no role in the overall cellular responses to cold (Cossins *et al.*, 1978; Gibson *et al.*, 1985). The inference, therefore, is that homeoviscous adaptation is not a property of all cellular membranes, but occurs selectively in situations where the compensation of membrane order leads to compensated membrane function, and this leads to some improvement in tissue function. This implies a very sophisticated regulatory control of membrane lipid composition and bilayer order by cells, and favors the idea of a cost–benefit relationship.

The expectation of ideal compensations may be unrealistic for another reason. Thus, membrane function is influenced by factors other than bilayer order, and homeoviscous adaptation should be viewed as only one of a broad cellular repetoire of adaptive responses (Cossins and Bowler, 1987). For example, the volume density and protein content of mitochondria increase

during cold acclimation, both of which enhance cellular metabolic activity by reducing the diffusion pathway for small molecules in the cytosol (Sidell, 1983; Sidell and Hazel, 1987). The combination of homeoviscous adaptation with these other cellular responses may well lead to more significant compensations in particular functional properties.

In contrast to thermal acclimation, the interspecific comparisons of brain membranes yielded HE's of 0.8–1.0 to both temperature and hydrostatic pressure; that is, the bilayer order of these membranes, as revealed by DPH anisotropy, was essentially identical at their respective habitat conditions. Thus, the potential for homeoviscous adaptation in the hands of evolution far exceeds that of any one species. This may not be too surprising since each species may become specifically adapted to a well-defined and restricted set of environmental conditions, rather than displaying the more flexible response of the acclimating animal. The fact that DPH anisotropy under adapted environmental conditions is so similar in fish, birds, and mammals suggested that the conserved bilayer order is of fundamental importance to the functional properties of brain membranes. However, the observation of ideal evolutionary compensation is presently restricted to brain membranes since interspecific studies of other membrane fractions, including sarcoplasmic reticulum and intestinal mucosa (J. A. C. Lee, personal communication), reveal no comparable pattern. The tissue organization and precise functional characteristics of these membrane fractions may vary from one species to another, depending upon differences in the predominant types of muscle fiber and gut organization of each species, so that the resulting membrane fractions are not strictly comparable in either functional properties or structure.

Finally, homeoviscous responses can only have adaptive significance through the modification of the functional properties of biological membranes. It is, therefore, worth asking whether membrane functions are, indeed, adapted to varying temperatures and pressures and to what extent these adaptations are related to homeoviscous responses. There is a substantial body of knowledge that indicates an important effect of bilayer order upon a number of functional properties of both artificial bilayers and biological membranes (Quinn, 1981; Lenza and Castelli, 1985; Wrigglesworth, 1985) so that alterations of membrane order should produce changes in at least some important functional properties. In the case of pressure, there is only limited information of adapted function. For example, DeLong and Yayanos (1987) found a measure of pressure tolerance in the glucose transport system of barophilic bacteria, but the role of membrane order was obscure. Few attempts have been made to investigate processes that require intact cells for their expression, such as ion transport, membrane excitability, and synaptic transmission. In most species, such processes are very sensitive to pressure,

and some of these effects may be mediated by changes in bilayer order (Macdonald, 1984). Therefore, it seems essential for deep-sea animals to have evolved suitably adapted systems for function under high pressure. Unfortunately, erythrocytes of deep-sea fish species are irreversibly damaged by decompression during trawling (Shelton *et al.*, 1985) and this has prevented a detailed analysis of pressure adaptation of transport function. Peripheral nerve and hearts from fish that normally live at 4000 m (40 MPa) function poorly at atmospheric pressures, but may be resuscitated by restoring the isolated preparation to the hydrostatic pressure normally experienced by the animal (Harper *et al.*, 1987; Pennec *et al.*, 1987). Again, the roles of membrane lipids and homeoviscous adaptation in these instances remain to be firmly established. Nevertheless, there is sufficiently good evidence that pressure influences $(Na^+ + K^+)$ATPase function indirectly via changes in membrane order (Chong *et al.*, 1985) to make this a likely possibility.

The physiological adjustments underlying thermal acclimation are more amenable to intimate attention and a sizable literature exists to indicate that membrane adaptation plays an important role in the overall cellular response (Hochachka and Somero, 1984; Cossins and Bowler, 1987). For example, the compensation of the oxidative capacity of mitochondria has been frequently observed (Hazel and Prosser, 1974). Part of this is due to adaptive changes in the membrane concentration of respiratory enzymes (Sidell, 1983) while membrane order also exerts a modulative role on the respiratory enzymes. Thus, Hazel (1972) found that reactivation of delipidated succinate dehydrogenase was greater with phospholipids from muscle of cold-acclimated goldfish than from warm-acclimated goldfish. Wodtke (1981) found significant increases in the turnover number of cytochrome *c* oxidase that were attributed to changes in the "viscotropic" environment of the enzyme, consistent with the idea of homeoviscous adaptation. With membrane permeability, Hazel and Shuster (1979) found that liposomes prepared with phospholipids from the liver of cold-acclimated trout were substantially more permeable than the corresponding liposomes of warm-acclimated trout. The $(Na^+ + K^+)$-stimulated ATPase of brain synaptic membranes showed a significant increase in thermal stability following warm acclimation, probably due to the restriction on protein conformational flexibility by the more ordered phospholipid environment (Cossins *et al.*, 1981). Smith and Ellory (1971) showed a change in the turnover number of $(Na^+ + K^+)$-stimulated ATPase of goldfish intestinal mucosa. The catalytic activity of this enzyme is not necessarily a good indicator of its transport function. Raynard (1988) has recently demonstrated the compensation of the molar activity of the Na^+ pump of erythrocytes from temperature-acclimated trout by using isotope flux analysis. The number of pump molecules, as determined by ouabain binding, was unaffected by acclimation, suggesting a role of homeoviscous

adaptation in regulating the transport function of this important enzyme. The sensitivity of the enzyme to membrane order was suggested by the inhibitory effect of cholesterol supplementation, and the consequent membane ordering, upon Na^+ pump activity. Thus, the evidence in favor of an important role of membrane order in the adaptation of membrane function is accumulating, though the precise details of the relationship between polypeptide flexibility and the mobility of the surrounding lipid matrix as dictated by the changing fatty acid saturation remains to be clearly established.

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