transport via the ASC system is important to cell function in terms of supplying precursors for intracellular glutathione biosynthesis. The observations of the temperature-sensitivity of the Na\(^{+}\)-pump, mediated passive K\(^{+}\) transport and amino acid transport systems confirm the heterogeneity of behaviour of different membrane transport systems in both hibernator and non-hibernator membranes (Ellory & Willis, 1981). Such results are important in emphasizing the improbability of a role for bulk membrane lipid effects in hibernation. Physical measurements addressed to determining membrane fluidity via fluorescence or n.m.r. techniques, compositional studies and detailed Arrhenius plots of individual membrane-bound enzyme activity have all been used to support the idea that bulk changes can occur in hibernator membranes (see Willis et al., 1981, for a review). The analogy with data from fish (see Cossins, 1983) and microorganisms (see Russell, 1983) is obvious and compelling. However, for the reason outlined at the outset, periodic arousal, and from the individuality of the temperature-dependence of the various transport systems presented here, we would conclude that at least the membrane microenvironment, and possibly the transport protein itself, is the site of adaptation in hibernator cold-tolerance. Recent reviews of epithelia have focused on the importance of the balance between pumps and leaks in terms of regulated intra-erythrocyte glucose (Schultz, 1981; Diamond, 1982). Thus in the present context what may matter in the end is whether hibernators have intrinsically less temperature-sensitive transport systems than do non-hibernators, but whether they co-regulate active and passive pathways to achieve a balance at both high and low temperatures.

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Adaptive responses of fish membranes to altered environmental temperature

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The dynamic structure or 'fluidity' of biological membranes appears to be subject to homeostatic control. The most persuasive evidence in support of this statement comes from the observation of homeostatic responses in the membranes of various animals, plants and micro-organisms when temperature is altered (Cossins, 1981). The physiological importance of this so-called 'homeoviscous adaptation' lies in the compensation of those membrane-associated processes and functions that are influenced by membrane fluidity for the direct effects of temperature changes. Although it is not possible to use this approach to demonstrate similar phenomena in homoeothermic animals (mammals and birds), there are several reasons for believing that homeoviscous adaptation occurs in these animals in response to nutritional or drug treatment (Cossins & Sinensky, 1983).

The main problem in studies of this sort is the biophysical measurement of membrane fluidity. Perhaps the most appropriate technique is fluorescence polarization, in which a hydrophobic fluorescence probe such as diphenylhexatriene is partitioned into the bilayer and is excited with vertically polarized light. The degree of polarization of the fluorescence reflects the degree of rotation of the rod-shaped probe during its excited lifetime (Pesce et al., 1971). Since the rotational characteristics of the probe are hindered and largely determined by molecular motion of the neighbouring hydrocarbon chains, it follows that this technique gives an index of membrane fluidity (Cossins, 1980, 1981).

The adaptation of fluidity may be observed by comparing the fluidities of membranes from cold- and warm-acclimated animals. This comparative approach largely overcomes some of the interpretive problems involved in the use of spectroscopic probes. Membrane preparations from cold-acclimated individuals tend to be more fluid than the corresponding preparations of warm-acclimated individuals. These differences are usually highly reproducible, indicating that fluidity is a finely regulated parameter. However, the adaptive response is certainly not 'ideal' or 'perfect' in the sense that membrane fluidity is held independent of seasonal changes in temperature. In fact, fish membranes only offer 50%, at best, of the temperature-mediated effects on membrane fluidity (i.e. homeoviscous efficacy = 0.5; Cossins, 1981). By comparison, the efficacy of bacterial membranes varies between 0.2 and 1.0, and for Tetrahymena between 0.1 and 0.4 (Cossins, 1981).

More recently, we have examined the homeoviscous efficacy of different membrane preparations isolated from goldfish brain (Cossins & Prosser, 1982). The myelin fractions was hardly affected by thermal acclimation, whereas the synaptic and mitochondrial fractions were significantly altered. This experiment indicates that different membrane types are affected by homeoviscous adaptation to quite different extents, though in the absence of completely pure membrane preparations it is still not possible to define unequivocally the efficacy of any particular membrane type.

The time-course of homeoviscous adaptation in fish appears to be rather slow (Cossins et al., 1977) compared with Tetrahymena and bacteria (see Russell, 1983). In the one study published so far, homeoviscous adaptation of brain membranes does correlate reasonably well with the time-course of resistance adaptation, which in fish appears to be a property of the central nervous system. The time taken for fluidity to reach a new steady-state value was found to be 10–14 days after transfer from 5°C to 25°C, but 30–50 days for the reverse.
Transfer. Tetrahymena and bacteria show adaptations that occur over a period of a few hours rather than days. This difference between fish and cultured organisms presumably is related to differences in their growth rates.

The correlation between the fluidity of synaptosomal preparations and cell/accumulation temperature may be successfully extrapolated to include other fish species from diverse thermal environments (Arctic Sculpin, 0.5°C, and Desert Pupfish, 34°C) as well as small mammals (37°C). Thus, interspecific differences in the fluidity of this membrane fraction appears to be related to differences in cell temperature rather than to other evolutionary factors (Cossins & Prosser, 1978).

Phospholipid liposomes prepared from 5°C- and 25°C-acclimated goldfish brain synaptosomes, and from rat brain synaptosomes, showed similar differences in viscosity/temperature profiles as observed for the native membrane (Cossins, 1977). The acclimation-dependent differences in membrane fluidity therefore appear to be mediated by shifts in the lipid composition of the membranes. In general, cholesterol content or phospholipid/cholesterol ratio is not affected, though the degree of unsaturation of membrane phospholipids is invariably increased as acclimation temperature is lowered (Cossins, 1977).


Adaptation to temperature in bacterial membranes

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Bacteria are poikilothermic organisms that do not possess mechanisms for maintaining a constant internal temperature. Moreover, they are small in size without insulating layers of fat so that changes in temperature must be sensed throughout the cell virtually instantaneously. Considered as a group, the bacteria occupy environmental niches ranging from the frozen tundra to boiling volcanic springs. In addition, the growth temperature range of a single species may vary enormously: some bacteria are unable to grow above 20-25°C (psychrophiles), whereas other cannot grow below 50-70°C (thermophiles) (Kushner, 1978). They may also have to adapt to wide seasonal variations in temperature. Even the familiar Escherichia coli and other human or animal pathogens, which are mesophiles growing well at 30-37°C and often at a relatively constant temperature, are capable of growing over a wide range of temperatures (e.g. 8°C for E. coli).

One of the most striking features of bacteria growing at different temperatures is the variation in the composition of their membrane lipids. It is these changes and the mechanisms by which they are achieved that form the basis of this review, together with a consideration of whether they are truly required for the adaptation to altered growth temperatures.

Bacterial classification and lipid composition

For the benefit of readers unfamiliar with bacterial lipids I will review briefly the lipid composition of the major groups of bacteria.

Recent studies, particularly of RNA sequences, have revealed the presence of not one but two phylogenetically distinct groups of bacteria, the 'true bacteria' (eubacteria) and the archaebacteria (Woese et al., 1978). These groups differ from each other as much as they both differ from Eukaryotes. As we shall see, one of the most striking differences between Eubacteria and Archaebacteria is the nature of their membrane lipids.

The major lipids in eubacteria are phospholipids, the most common being phosphatidylglycerol, phosphatidylethanolamine and diphosphatidylglycerol (cardiolipin) (Goldfine, 1982). Phosphatidylethanolamine is often the predominant phospholipid in Gram-negative bacteria, whereas Gram-positive bacteria contain relatively more phosphatidylglycerol and cardiolipin. Phospholipids are present in both the cytoplasmic membrane and the outer membrane of Gram-negative bacteria, which also have lipopolysaccharide in their outer membrane. Bacterial membranes lack sterols, but do contain glycolipid, usually in small amounts; in those few species (e.g. Streptococci) where glycolipids represent a significant proportion of the total lipid, the main component is glucosyldiacetylglucosylceramide. The major fatty acids of bacteria are saturated and monounsaturated fatty acids with 14-20 carbon atoms, and they are attached to the glycerol backbone of phospholipids and glycolipids by ester linkages. Gram-negative-bacterial lipids contain even-numbered straight-chain fatty acids, whereas those of Gram-positive bacteria contain odd-numbered fatty acids, which may have methyl branches, either (so [CH3-CH(CH3)-CH2]- or anteiso [CH3-CH2-CH(CH3)-CH2]-). The lipopolysaccharide of Gram-negative bacteria contains a proportion of 2- or 3-hydroxy fatty acids, which are not normally present in the phospholipids.

Archaebacteria also contain phospholipids, but in complete contrast they have largely C20 phytanoyl chains (saturated isoprenoid-derived) with repeating methyl branches in ether linkage to the glycerol backbone (Kates, 1978; Kushner et al., 1981). In addition, in halophiles the head groups are often more negatively charged, bearing extra phosphate or sulphate residues. The phytanoyl chains of one phospholipid may be covalently linked to those of another, producing a C60 tetraether lipid that can span the membrane width. Some thermoalkaliphiles also have C32 sesterpenyl chains, and some thermoacidophiles have one or four cyclopropane rings along the alkyl chain. The various C20, C4, and C8 chains give a variety of possible structures for the hydrophobic core of the membrane (De Rosa et al., 1982, 1983).

Effect of bacterial growth temperature on lipid composition

Although there are some exceptions, most bacterial species change the fatty acid composition of their lipids in response to variations in environmental temperature (Heinrich, 1976). Changes in phospholipid head-group composition are much less frequently observed and are often quantitatively unimportant. There being no membrane sterols, changes in the sterol/