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Effects of Temperature on Lipid Unsaturation

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Introduction

In nature and in the laboratory, a decrease in temperature may cause liquids to solidify and an increase in temperature may cause solids to liquefy. These gel/liquid crystalline phase transformations and concomitant changes in membrane fluidity can be measured in bulk by various techniques including fluorescence polarization, differential scanning calorimetry, electron spin resonance and spin labelling. The integrity of living cells in response to environmental stresses such as temperature depends very much on the bimolecular lipid layer and the associated non-lipid components. The *in situ* properties of these lipids can likewise be monitored by techniques already noted (Cossins, 1977; Brenner, 1984; McElhaney, 1984; Connolly *et al.*, 1985; Raison, 1985; Reizer, Grossowicz and Barenholz, 1985; Stocker *et al.*, 1987). Lipid molecules in the bilayer normally have a degree of mobility, thereby behaving as a two-dimensional liquid rather than a solid. Depression of temperature can decrease this mobility and simultaneously decrease the vital activities of the cell, but these changes do not necessarily cause death without other physiological changes.

Examination of lipids from widely varying organisms, from bacteria to vertebrates, shows that the fluidity of the lipids, whether of cell membranes or depot fats, is related to 'body' temperature. In all instances the lipids are fluid at temperatures at which the organism normally exists. In general, the higher this temperature, the higher the lipid melting point; the lower the temperature, the lower the lipid melting point.

Living systems have evolved response networks, homeoviscous or homeophasic adaptations, to maintain appropriate membraneous liquidity or phase relationships (McElhaney, 1984). The necessary structural adaptations can be brought about in a variety of ways. Degree of fatty acid unsaturation is perhaps the most prevalent but others can play a major part, notably vari-

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ation in chain length, branching and cyclization of fatty acids as well as the distribution and relative proportions of glycolipid, phospholipid and other lipid families.

It is not known whether the effects of temperature act directly on genetic or biochemical expression or whether, at least in some instances, the temperature effect is indirect via cell growth rate or oxygen availability, for instance.

This review deals especially with the relationship between temperature and lipid unsaturation but some ancillary topics are briefly mentioned. This is because temperature effects are seldom exerted separately from other related factors that can also affect lipid composition: these may include nutrient availability, pH, culture age, growth rate, oxygen tension, pressure, salinity, drought, humidity and genetic or biochemical competence.

Knowledge of the factors affecting relations between lipid composition and temperature is of great importance in planning work intended to modify organisms to produce lipids of desirable compositions. Genetic manipulation of plants offers promise in modifying the composition of fats from oil seed to generate highly desirable and specialized products. Until more is known of the restraints imposed by temperature, however, it may not be possible to select rationally the best species for use or the most appropriate climatic zone for growth. Information on the lipids at cell surfaces, the enzymes that produce and modify them and the additional enzymes that operate in the lipid milieu could well be of value in other applications. For instance, losses suffered by crops under extreme temperatures may depend on lipid compositions, as may the adverse effects suffered by tropical fruits during cold storage. Such research could also be useful in extending knowledge of enzymic activity in organic solvent systems.

Temperature and lipid unsaturation in micro-organisms

The lion's share of the experimental data linking temperature and lipid unsaturation has been generated in studies on micro-organisms, with lesser information from plants and animals. A number of excellent reviews are available (Herbert (1981), Langworthy (1982), Ratledge (1982) and Russell (1984) on microbial systems; Thompson (1983, 1985) on agricultural plants), but none of these have attempted to cover the three classes of living systems as well as isolated enzymes in one paper. An overall review is the purpose of this chapter.

Data illustrating the increase in unsaturated fatty acids with decreasing temperature in phospholipids of *Neurospora crassa* (Vogt and Brody, 1985) and *Paecilomyces persicinus* (Parmegiani and Pisano, 1974) are shown in *Tables 1–2*. There were both differences and similarities, representative of species-to-species variation. The major similarities were the considerable decrease in 18:1 (oleic acid) and the marked increase in 18:2 (linoleic acid) + 18:3 (linolenic acid) with lower temperature, where the number after the colon represents the number of double bonds in the fatty acid. The differences were that in *Neurospora* the major increase was in 18:3 while with *Paecilomyces* the major increase was in 18:2.

Table 1. Effect of temperature on fatty acid unsaturation in phospholipids of *Neurospora crassa*

Fatty acids	Percentage of total fatty acids					
	22°C	29°C	32°C	36°C	40°C	% 22°C − % 40°C
16:0	20	21	23	21	23	−3
18:0	3	4	4	3	3	0
18:1	5	12	15	24	28	−23
18:2	45	46	44	43	38	+7
18:3	27	17	14	9	8	+19

Table 2. Effect of temperature on fatty acid composition of phospholipids of *Paecilomyces persicinus*

Fatty acids	Relative %		
	20°C	36°C	% 20°C − % 36°C
16:0	22·7	14·3	+ 8·4
18:0	2·2	Trace	+ 2·2
18:1	9·6	66·9	−57·3
18:2	54·7	18·8	+35·9
18:3	10·8	Trace	+10·8

A more detailed look at the effect of temperature on a variety of fatty acids present in the total lipids of *Flavobacterium halmephilum* is shown in *Table 3* (Monteoliva-Sanchez and Ramos-Cormenzana, 1986). In this case, the major increase in fatty acids with lower temperature was not in more unsaturated compounds but in branched 15:0 and cyclopropane-19 carbon fatty acids. The major decreases in fatty acids occurred with 18:0 (stearic acid) and 20:0 (eicosanoic acid). This example shows that microbial systems have alternative mechanisms other than unsaturation by which they can achieve homeoviscous adaptation. Each system must be individually analysed to determine which response has been elicited.

Another example of the effect of temperature on lipid unsaturation is in the biosynthesis of wax esters by *Acinetobacter* sp. Ho1-N (Neidleman and Geigert, 1984). Wax esters are produced from long-chain fatty acids and alcohols, according to this simplified equation:

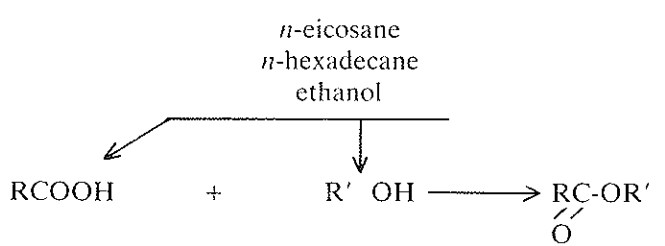

Table 3. Effect of temperature on fatty acids of *Flavobacterium halmephilum* CCM 2831

Fatty acids	Percentage of total fatty acids		
	22°C	42°C	% 22°C − % 42°C
14:0	0·45	None	+ 0·45
16:0	2·52	1·60	+ 0·92
17:0	1·77	None	+1·77
18:0	4·88	43·21	−38·33
20:0	None	25·55	−25·55
br-14:0*	2·83	None	+ 2·83
br-15:0	51·42	Trace	+51·42
br-16:0	3·62	None	+ 3·62
br-17:0	None	7·41	− 7·41
16:1	15·10	23·81	− 8·71
cyc-17**	3·61	None	+3·61
cyc-19	10·78	None	+10·78

*br = branched **cyc = cyclopropane

Table 4 indicates the following two points: (1) decreasing the temperature increased the percentage of diene wax esters and the overall unsaturation index; (2) the unsaturation index, while markedly affected by temperature, was also influenced by the nature of the substrate. The highest unsaturation index was obtained with *n*-eicosane, followed by ethanol and then *n*-hexadecane. These results illustrate that, in order to take maximum advantage of the intrinsic capacity of a biological system to form unsaturated and other alternative homeoviscous responses, temperature is not the only variable needing scrutiny; it is not necessarily an independent variable.

Table 5 expands this idea by presenting a few examples of variables other than temperature that cause increased lipid unsaturation in selected microorganisms. Particularly prominent in this listing are the nutrient-related factors such as glucose versus glycerol, N- and C-limited media, glucose versus arabinose and dilution rates. In addition, Ratledge (1982) emphasized the effect of oxygen tension on the lipid composition of various yeasts. In general, high O_2 availability was associated with an increase in lipid unsaturation at a given temperature. This is in agreement with the hypothesis of Harris and James (1969), who suggested that an increase in oxygen solubility and, therefore, availability at low temperatures, was the major reason for increased unsaturated lipid, because desaturation is oxygen-dependent. However, this concept continues to be controversial, as will be discussed below.

Additional examples of temperature-mediated effects on lipid unsaturation are given in *Table 6*. Some of the references cited indicate a number of other points that are worthy of special consideration.

Aaronson, Johnston and Martin (1982) reported that the distribution of phospholipids in *Neurospora crassa* was temperature dependent. Similar observations have been made in many other studies, for example, in *Absidia ramosa* (Raju, Maheshwari and Sastry, 1976), *Mucor mucedo* and *Aspergillus*

Table 4. Effect of temperature on unsaturation of wax esters produced from *n*-eicosane, *n*-hexadecane and ethanol by *Acinetobacter* sp H01-N.

Substrate	Temperature (°C)	Diene fractions (total)	Monoene fractions (total)	Saturated fractions (total)	Unsaturation index*
		(36:2, 38:2, 40:2)	(36:1, 38:1, 40:1)	(36:0, 38:0, 40:0)	
n-eicosane	17	17%	24%	5%	1·66
	24	41%	37%	22%	1·19
	30	35%	40%	25%	1·10
		(32:2)	(32:1)	(32:0)	
n-hexadecane	17	15%	35%	50%	0·65
	24	8%	31%	61%	0·47
	30	2%	18%	80%	0·22
		(32:2, 34:2, 36:2)	(32:1, 34:1, 36:1)	(32:0, 34:0, 36:0)	
Ethanol	17	72%	18%	10%	1·62
	24	29%	40%	31%	0·76
	30	9%	25%	66%	0·43

* (% monounsaturated + 2 × % diunsaturated)/100.

Table 5. Effect of variables other than temperature on lipid unsaturation of micro-organisms

Micro-organism	Type*	Variables compared	Lipid class or source	Major effect	Reference
Microsporum gypseum	F	Glucose vs glycerol	Total phospholipids with glucose	> Unsaturation	Jindal *et al.*, 1983.
Rhodotorula gracilis	Y	N-limited vs C-limited	Total phospholipids	> Unsaturation with N-limitation	Rolph *et al.*, 1983.
Taphrina deformans (DI)	F	Control vs triazole antifungal CGA-64250	Total lipids	% 18:3 increased by triazole	Weete, Sancholle and Montant, 1983.
Saccharomyces rouxii	Y	Control vs NaCl	Total fatty acids	% 18:2, 16:1 decreased and % 18:1 increased with increasing NaCl.	Watanabe and Takakuwa 1984.
Escherichia coli	B	Control vs ethanol and trichloroacetic acid	Total fatty acids	% 18:1 increased with ethanol and trichloroacetic acid % 16:0 decreased by ethanol	Ingram, 1982.
Rhizopus arrhizus	F	Glucose vs arabinose	Total fatty acids	% 18:3 > with arabinose	Gunasekaran and Weber, 1975.
Marine bacteria	B	Increasing pressure	Total fatty acids	General increase in % 20:5 and 22:6 with increasing pressure	DeLong and Yayanos, 1986.
Rhodotorula glutinis	Y	N- and C-limited, various dilution rates	Neutral, glyco- and phospholipids	Unsaturation greater in neutral and glycolipids in C-limitations; in N-limitations, phospholipid unsaturation greater. Unsaturation increased with increasing dilution rates.	Yoon and Rhee, 1983. Hansson and Dostalek, 1986.

*B = bacterium; F = fungus; Y = yeast.

Table 6. Effect of temperature on lipid structure and composition in micro-organisms

Micro-organism	Type*	Temperatures compared (°C)	Lipid class or source	Major effect	Reference
Hansenula polymorpha	Y	20° vs 50°	Total lipids and phospholipids	% 18:3 > 20° % 18:2, 18:1 > 50° phospholipid > 20°	Wijeyaratne *et al.*, 1986.
Microsporum gypseum		20° vs 27°	Total phospholipids	% 18:2 > 20° % 18:0 > 27°	Jindal *et al.*, 1983.
Neurospora crassa	F	15° vs 37°	Phospholipids of whole cells, mitochondria and microsomes	% phosphatidyl-ethanolamine > 15° % phosphatidyl choline > 37° % 18:3 > 15° % 18:2 > 37° % 16:0 > 37°	Aaronson, Johnston and Martin, 1982.
Bacillus stereothermophilus var. *nondiastaticus*	B	45° vs 65°	Total phospholipids	% branched fatty acids > 45° % saturated fatty acids > 65°	Reizer, Grossowicz and Barenholz, 1985
Emericellopsis salmosynnemata	F	20° vs 36°	Total phospholipids and triglycerides	% 18:2, 18:3 > 20° % 18:1 > 36°	Parmegiani and Pisano, 1974.
Candida lipolytica	Y	10° vs 25°	Microsomal membranes	% 18:2 > 10°	
Yersinia enterocolitica 755	B	10° vs 40°	Lipopolysaccharide	% 16:1, 12:0 > 10° % 14:0 > 40°	Wartenberg *et al.*, 1983.
Planococcus sp. (strain A4a)	B	5° vs 35°	Total fatty acids	% 16:1, 17:1 > 5° % 16:0, 17:0 > 35°	Miller, 1985.

*B = bacterium, F = fungus, Y = yeast.

ochraceus (Chavant, Sancholle and Montant 1979), *Bacillus caldotenax* (Hasegawa, Kawada and Nosoh, 1980), *Vibrio* and *Pseudomonas* (Herbert, 1981), *Sarcina marina* (Hunter, Olawoye and Saynor, 1981) and *Staphylococcus aureus* (Pisano, Ball and Eriquez, 1983).

It was mentioned above that *Flavobacterium halmephilum* illustrated an alternative response to lower temperature—an increase in branched chain fatty acids. Reizer, Grossowicz and Barenholz (1985) reported a similar activity in *Bacillus stearothermophilus*, a thermophilic bacterium. Pond and Langworthy (1987) studied a thermophilic eubacterium, *Thermomicrobium roseum*, in which long-chain diols and fatty acids showed increased branching as growth temperature was reduced from 75°C to 60°C; there was also a small increase in chain length. The authors also mention that thermophiles may respond to lowering temperatures by decreasing the ratio of iso- to anteiso branched-chain fatty acids. The general response of all micro-organisms to reduced temperature is to synthesize lipids with lower melting points. This may be achieved by unsaturation, branching, cyclization and chain shortening.

It is tempting to suggest that at least one reason for the thermophilic characteristic of these bacteria is the inability to synthesize unsaturated fatty acids to allow growth at lower temperatures. Wright, Kafkewitz and Somberg (1983) suggested such an explanation for the thermophile *Talaromyces thermophilus* by stating that the metabolic limitations that restrict its ability to regulate membrane fluidity relegate the micro-organism to the thermophilic life. Cold water can be figuratively dashed on this simple view by the reminder of McElhaney (1976) that merely altering the membrane lipids of a bacterium such as *Escherichia coli* would not convert it to a psychrophile because a key protein may yet be cold-denatured. It is, however, relevant to note that in many studies (Hammonds and Smith, 1986) the profile of lipid unsaturation was highest in psychrophiles, followed by mesophiles and, lastly, thermophiles, in obvious relationship to their preferred growth temperatures.

Nevertheless, there are examples in which a direct rather than inverse relationship exists between lipid unsaturation and temperature. Bhakoo and Herbert (1980) reported that, in five psychrophilic marine *Pseudomonas* sp., decreasing the growth temperature from 20°C to 0°C resulted in little significant change in fatty acid composition. It is easy to dismiss this result as an example wherein the high level of lipid unsaturation associated with these organisms at any growth temperature already exceeds the necessity for membrane fluidity at 0°C, and, therefore, no change is required. It is not as easy to ignore the cases in which lipid unsaturation increases with increasing temperature, as in a number of studies with fungi (Ratledge, 1982). A particularly dramatic example of this response is seen in *Table 7* from the work of Gunasekaran and Weber (1975) on the fatty acids of the polar lipids of *Rhizopus arrhizus*. It can be seen that the major effects of reducing the growth temperature were a reduction in unsaturation and an increase in shorter-chain fatty acids; the two responses were roughly equal in magnitude. This may represent a case in which maintenance of membrane fluidity

Table 7. Effect of temperature on fatty acids of polar lipids of *Rhizopus arrhizus*

Fatty acids	Percentage of total fatty acids		
	15°C	30°C	% 15°C − % 30°C
14:0	26·21	1·89	+ 24·32
14:1	19·47	0·64	+ 18·83
15:0	4·54	0·72	+ 3·82
16:0	11·27	14·36	− 2·09
16:1	Trace	1·04	− 1·04
18:0	Trace	3·54	− 3·54
18:1	17·28	26·06	− 8·78
18:2	8·42	25·69	− 17·27
18:3	12·81	26·05	− 13·24
Total (14:0 + 14:1)	45·68	2·43	+ 43·25
Total (18:1 + 18:2 + 18·3)	38·51	77·80	− 39·29

through homeoviscous adaptation at lower temperatures is achieved with smaller fatty acids rather than unsaturated ones—a deviant but not unsupportable conclusion. A closer look at the data for *Paecilomyces persicinus* (*Table 2*) indicates an increase in 16:0 (palmitic acid) at 20°C compared with 36°C, perhaps a similar response although to a lesser degree. Results with *Bacillus licheniformis* (Jindal *et al.*, 1983; Russell, 1983) also indicated that a decrease in carbon chain length of fatty acids was one response to low temperature. The data of these latter three reports were modest, however, in comparison to those of Gunasekaran and Weber (1975).

The final point to be emphasized from *Table 6* is that the lipid incorporated into the lipid A component of lipopolysaccharides of not only *Yersinia enterocolitica*, as indicated in the Table, but also of *Salmonella* strains, *Escherichia coli* and *Proteus mirabilis*, was also influenced by temperature. In all cases, the level of 16:1 (palmitoleic acid) was increased at lower temperatures at the expense of either 12:0 (dodecanoic acid) or 14:0 (myristic acid), depending upon the species or strains.

This brief discussion of the effects of temperature on lipid unsaturation in micro-organisms is not altogether straightforward because, although a major response is to increase unsaturation with decreasing temperature, exceptions and alternatives are not unknown. However, a good rule is to expect this response but not to be surprised at the occurrence of an alternative. In most studies on micro-organisms, absolute measurements on membrane fluidity and lipid melting/freezing points have not been obtained and so the absence of an effect may occasionally simply represent the fact that the lipid already has the desired characteristics for survival. Many aspects of the interplay of variables such as temperature, oxygen availability, nutrients, stage of growth, and dilution rate are still unknown and unpredictable and may be species specific. The mechanisms involved in some of these responses are discussed below after consideration of temperature and unsaturation in plants and animals.

Temperature and lipid unsaturation in plants

As with micro-organisms, one of the major effects of reducing growth temperature in plant systems is an increase in lipid unsaturation, with increasing growth temperature causing a decrease in lipid unsaturation. Only a few of the many reported examples will be mentioned here.

In *Table 8* are shown data for the level of various fatty acids present in the leaf tissue of the tree, *Ceratonia siliqua*, as a function of the seasons (Diamantoglou and Meletiou-Christou, 1980). These 'natural' data indicate that the highest levels of the 18:2 and 18:3 fatty acids were reached during November–June, while the lowest concentrations were obtained during July–October. With the less saturated fatty acids 16:0, 18:0 and 18:1, the reverse was noted: the highest levels were found during July–October and the lowest levels from November to June.

Sanders (1982) studied the effects of geographical location and temperature on the unsaturation of triacylglycerol in American peanut (*Arachis hypogea*) oil; the data are given in *Table 9*. The mol% (percentage on a molar basis) of 18:1 always exceeded that of 18:2, but it is clear that this difference was diminished by growing the peanuts at cooler temperatures. In a similar study investigating the effects of geographical location and temperature on unsaturation in Japanese sunflower (*Helianthus annuus*) seed oil, Nagao and Yamazaki (1983) reported the data shown in *Table 10*. In this case, in contrast to the American peanut oil, the percentage of 18:2 always exceeded that of 18:1; however, in agreement with the results of the peanut study, lower temperatures favoured 18:2 production and, therefore, increased the difference. At 27·4°C the percentages of 18:2 and of 18:1 fatty acids were almost equal, whereas at 19·4°–19·7°C, the level of 18:2 was ~60% greater than that of 18:1.

These three studies, carried out in the field, strongly support the premise that increased unsaturation is favoured by decreasing temperature. Although secondary variables such as oxygen availability, as a function of temperature and nutrient differences, may play a part, temperature appears to be the

Table 8. Effect of temperature on fatty acids of *Ceratonia siliqua* leaves

	Percentage of total fatty acid											
	1975						1976					
Fatty acid	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June
<14:0	13·0	12·6	10·9	9·2	14·3	6·8	6·1	3·9	3·4	7·7	7·5	9·6
16:0	25·2	28·7	25·6	25·1	17·7	21·5	19·5	19·4	17·9	18·3	17·9	18·1
16:1	1·6	T*	T	T	T	T	T	0·9	0·5	0·8	0·9	0·8
18:0	4·9	6·0	4·7	4·9	3·9	4·1	4·5	4·4	4·6	4·2	4·1	3·8
18:1	14·6	14·4	15·4	14·8	8·6	5·9	5·8	5·1	4·2	4·9	6·6	6·1
18:2	10·3	8·4	8·4	9·4	12·3	11·8	11·7	13·5	12·5	10·2	11·3	10·9
18:3	29·5	28·4	33·3	34·9	41·4	48·2	50·6	51·2	54·4	51·9	50·3	49·6

*T = trace.

Table 9. Effect of temperature on unsaturation in American peanut oil triacylglycerol

Variety	Location	Temperature *(°C)	mol % 18:1	mol % 18:2	mol % difference 18:1 − 18:2
Florunner	Gainesville, FL	26·6	52·7	29·7	23·0
Florunner	Headland, AL	25·5	50·9	29·1	21·8
Florunner	Suffolk, VA	21·7	46·1	35·9	10·2
Florunner	Stephenville, TX	14·7	44·5	37·4	7·1
Florigiant	Gainesville, FL	26·6	54·8	26·1	28·7
Florigiant	Headland, AL	25·5	53·1	27·3	25·8
Florigiant	Suffolk, VA	21·7	45·9	34·7	11·2
Florigiant	Stephenville, TX	14·7	44·7	36·7	8·0

* mean temperatures for 6 weeks preceding harvest.

Table 10. Effect of temperature on unsaturation in Japanese sunflower seed oil

Variety	Location	Temperature (°C)*	Percentage of total fatty acid 18:0	18:1	18:2	Percentage difference 18:2 − 18:1
Tainan #1	Gifu	23·5	3·9	28·3	62·1	33·8
Tainan #1	Gifu	20·4	4·5	22·1	67·6	45·5
Tainan #1	Okayama	27·4	3·0	44·7	46·4	1·7
Tainan #1	Okayama	19·4	4·2	14·3	74·5	60·2
IS-903	Okayama	27·4	3·4	45·0	45·8	0·8
IS-903	Okayama	19·7	5·1	14·8	73·5	58·7
IS-907	Okayama	27·4	2·6	44·9	46·4	1·5
IS-907	Okayama	19·7	3·7	13·6	75·4	61·8
IS-907	Ehime	26·5	2·6	42·9	48·7	5·8
IS-907	Ehime	23·5	3·0	29·4	61·3	31·9

*Average temperature during seed maturation

primary determinant. The growth of other crops, for example soybeans (*Glycine max*) (Martin, Wilson and Rinne, 1986), and flax (*Linum usitatissimum*) (Green, 1986), for seed oil production under controlled field conditions, also indicates an increase in lipid unsaturation at low temperatures. In both of the studies cited, the authors stated that oxygen availability was not responsible for the effects that were obtained; this is in contrast to Harris and James (1969), who concluded that the increase in desaturation activity at lower temperatures in sunflower and castor bean (*Ricinis communis*) seeds was due to greater oxygen availability which, at higher temperatures, is the rate-limiting factor for desaturation. In contrast, Wolf *et al.* (1982) in studies on soybean plants supported the hypothesis of Harris and James, but based this support on theoretical principles rather than hard data.

Other plant systems yielding data supporting the inverse relationship of temperature and lipid unsaturation include asexual embryos of cacao (*Theo-*

broma cacao) (Wright, Janick and Hasegawa, 1983), tobacco (*Nicotiana tabacum*) cell suspensions (Gawer, Sansonetti and Mazliak, 1983), algae (*Chlorella minutissima, Fucus vesiculosus, Phycodrys sinuosa, Porphyridium cruentum*) (Pohl and Zurheide, 1979; Seto, Wang and Hesseltine, 1984; Lee, Tan and Tan, 1985), pea (*Pisum sativum*) plants (Chapman and Barber, 1981), and rape (*Brassica napus*) and wheat (*Triticum aestivum*) seeds (Mazliak, 1979). *Table 11* illustrates data for callus cultures of rape and nasturtium (*Tropaeolum majus*) (Mangold, 1977). In both cases, the response to lower temperature was to decrease fatty acids type 16:0 and 18:1 and to increase 18:2 and 18:3.

It should be emphasized that, in plants as well as in micro-organisms and animals, data on the effect of temperature on lipid unsaturation for a particular species may be confusing because results may vary depending upon whether total lipids, particular lipid classes, specific membrane preparations or tissues of different ages, for example, are the experimental material under analysis. Bartkowski (1979) investigated temperature effects on lipid unsaturation in nuclear, mitochondrial and microsomal membranes of cotton (*Gossypium barbadense*) seed and found that the major increase in unsaturation at low temperatures occurred in the microsomal membranes. Chapman and Barber (1981) emphasized that while both thylakoid and non-thylakoid membranes showed increased unsaturation with decreased temperatures, the details of the effects were very different depending upon the nature of the membrane. Osmond, Wilson and Raper (1982) reported that the response to low temperatures of fatty acid composition in old and new roots of soybeans was influenced by tissue ageing or differentiation.

In plant systems there are two major reasons for commercial interest in the relationship between temperature and lipid unsaturation. The first is that the chemical composition of the agricultural product can be strongly influenced by the temperature at which the crop is grown. The level of fatty acid unsaturation has both positive and negative aspects. From a health point of view, unsaturated fatty acids are generally considered to be desirable, but, from a product quality viewpoint, unsaturated fatty acids such as 18:3 can be undesirable because of their instability in processing, storage and use

Table 11. Effect of temperature on the fatty acids in callus cultures of rape and nasturtium

	Percentage of total fatty acids					
	Rape			Nasturtium		
Fatty acids	5°C	30°C	%5°C – %30°C	5°C	30°C	%5°C – %30°C
16:0	21·6	27·6	−6·0	25·6	27·1	−1·5
18:0	5·6	7·2	−1·6	trace	trace	—
18:1	12·8	19·0	−6·2	24·0	35·3	−11·3
18:2	24·0	22·6	+1·4	24·1	14·6	+9·5
18:3	32·8	17·6	+15·2	26·3	23·0	+3·3

(Raison, 1985; Green, 1986). The second reason is that the sensitivity of agricultural crops to frost damage is thought by many researchers to be related to the content of unsaturated lipids, higher levels of unsaturation favouring hardiness to low temperatures (Kimura *et al.*, 1982; Horvath *et al.*, 1983; Christiansen, 1984; St John, Christiansen and Terlizzi, 1984). However, this relationship is still controversial (Willemot, 1979; Maluf and Tigchelaar, 1982; Harwood, 1984). Nevertheless, considerable research effort has been devoted to genetic improvements and chemical treatments to create more hardy crop plants.

Temperature and lipid unsaturation in animals

The most intensively studied member of the animal kingdom, from the point of view of lipids, is the ciliated protozoon *Tetrahymena pyriformis* NT-1. *Table 12* presents data representative of the temperature effect on lipid unsaturation in this creature. It can be seen that the major effects on the fatty acids of the total lipid were an increase in short chain 14:0 and 16:1 compounds and unsaturated 18:2 fatty acid and decreases in less unsaturated acids 16:0, 18:0, 18:1 and 19:0 (nonadecanoic acid). The effects on the fatty acids of phospholipids were similar except for a slight decrease in 14:0 rather than an increase (Connolly *et al.*, 1985). In the sphingolipid subset of the phospholipids of the surface membranes a variant effect of temperature on lipid structure was reported by Kaya, Ramesha and Thompson (1984). At 39°C the synthesis of both 16:0 and 9-OH 16:0 acids was favoured, while at 15°C the primary fatty acids produced were 16:0 and 17:0 (heptadecanoic acid). These hydroxylated fatty acids offer the less rigid, increasingly fluid membrane property, as do the unsaturated, branched, cyclized and short-chain fatty acids.

In addition to these effects on fatty acids, the distribution of polar head groups, which determine the specific phospholipid or glycolipid, was tempera-

Table 12. Effect of temperature on fatty acid unsaturation of total lipids and phospholipids of *Tetrahymena pyriformis* NT-1

	Percentage of fatty acids					
	Total lipids			Phospholipids		
Fatty acids	20°C	38°C	20°C − 38°C	20°C	38°C	20°C − 38°C
14:0	22·0	15·5	+6·5	9·2	10·8	−1·6
16:0	19·7	22·8	−3·1	18·0	20·0	−2·0
16:1	9·0	2·1	+6·9	8·3	3·2	+5·1
18:0	2·4	8·3	−5·9	1·2	2·3	−1·1
18:1	5·1	10·3	−5·2	10·2	13·9	−3·7
18:2	21·7	16·2	+5·5	25·6	22·9	+2·7
18:3	18·2	18·1	+0·1	23·1	22·6	+0·5
19:0	0·4	4·1	−3·7	1·5	1·9	−0·4
20:1	0·4	2·1	−1·7	1·8	1·9	−0·1

ture responsive in *Tetrahymena*. Growth at 15°C, in contrast to 34°C, resulted in a decrease in phosphatidylethanolamine, an increase in 2-aminoethylphosphonolipid, and no change in phosphatidylcholine. Shifts in polar head group structure in other living systems have also been reported, but different classes of phospho- and glycolipids were involved (Umeki, Maruyama and Nozawa, 1983; Thompson, 1985).

The molecular basis for the increased level of unsaturated fatty acid synthesis as a result of growth at, or temperature shifts to, low temperature (<20°C) is still a subject of considerable discussion in the case of *Tetrahymena*, as it is in other micro-organisms, plants and animals. Thompson (1979) argues against increased oxygen availability as an acceptable mechanism. Several authors suggest that two distinct mechanisms occur in *Tetrahymena*: (1) induced synthesis of new desaturase molecules; (2) the modulation of existing desaturase molecules by membrane fluidity (Thompson, 1979; Kasai and Nozawa, 1980; Kasai *et al.*, 1985; Thompson, 1985). Another mechanism has been postulated by Stern and Pullman (1983), who provided evidence of specific inhibition of fatty acid desaturase activity by increased levels of particular polyenoic fatty acids.

Although *Tetrahymena pyriformis* NT-1 is the major source of information regarding the relationship of temperature and lipid unsaturation in animals, there are many other relevant sources of useful, informative and, sometimes, befuddling data. These include studies with fish, rats and insects. Consideration of a few of these follows.

Patton (1975) reported that, in fish, reducing environmental temperature led to an increase in tissue lipid unsaturation. Phospholipids and triglycerides of white skeletal muscle from three tropical surface, three deep-water and one Antarctic species were examined. The surface species were living at 23–25°C and 101–505 kPa (1–5 atm) pressure, the Antarctic species at −1·9°C and 101–505 kPa and the deep-water species were at 2–4°C and 20200 kPa pressure. Compared with those of tropical surface fish, phospholipids of Antarctic and deep-water fish had higher levels of polyunsaturation and lower levels of saturation. Triglyceride fatty acids tended to be more unsaturated in cold-water species. One question that can be raised is whether these effects of temperature on unsaturation are directly on the fish or are merely a reflection of the level of lipid unsaturation in their dietary algae, which would be expected to show such differences. This is one of the difficulties in interpretation of effects, in one member of a complex life cycle wherein all the variables are neither understood, nor are controllable.

A study providing a clearer indication of the effect of temperature on lipid unsaturation in fish was reported by De Torrengo and Brenner (1976) who studied the ability of liver microsomes of *Pimelodus maculatus* to desaturate and elongate 18:1, 18:2, and γ-18:3 fatty acids. The authors found that fish kept at 14–15°C had a higher desaturation and elongation activity than those kept at 29–30°C. They also noted that earlier studies by other investigators had indicated a tendency in fish to increase the polyunsaturated 22:6 fatty acid (docosahexaenoic) and decrease 16:0 and 18:0 fatty acids at lower temperatures. In addition, Cossins (1977) reported that the fluidity of synap-

tosomal membrane preparations of cold (5–15°C)-acclimatized goldfish was greater than that from warm (25°C)-acclimatized goldfish. The increased fluidity was correlated with a decrease in the proportion of saturated fatty acids in major phospholipids and an increase in unsaturation in phosphatidylcholine.

From these data it seems reasonable to assume that fish may respond to decreased temperature by increasing the unsaturation in their lipids. The use of fish as experimental material in the study of temperature and unsaturation has been defended on the basis of their poikilothermic characteristics, as they must respond to such stresses in a more direct way than the homoiothermic mammals. In this regard it is particularly interesting that a study in rats on the regulation of Δ^6-desaturase (EC 1.14.99.5) and serum fatty acid composition at 15°C and 30°C showed results similar to those in fish.

Gonzalez, Nervi and Peluffo (1986) have shown that female rats kept at 30–32°C for 20–25 days developed an increased Δ^6-desaturation activity in microsomal membranes after a 5-day shift to 13–15°C (*Table 13*). Male rats and ovariectomized female rats did not show such an effect. Administration of 17β-oestradiol caused a decrease in Δ^6-desaturase activity in 15°C-adapted female rats suggesting that oestradiol levels may have a role in Δ^6-desaturase (acyl-CoA desaturase) regulation during cold adaptation. Additionally, the data in *Table 13* illustrate the effect of the temperature shift to 15°C from 30°C on fatty acid unsaturation in female rat serum. The major changes were decreases in 16:0 and 18:0 and an increase in 20:4 (arachidonic acid), very similar to the effects reported in the fish *Pimelodus maculatus*, as discussed above.

An example of a non-temperature-mediated increase in lipid unsaturation has been reported in the fatty acids obtained from phospholipids of erythrocytes and plasma of mice infected with *Plasmodium vinckei* (Stocker *et al.*, 1987). It was demonstrated that, in both the red blood cells and plasma, the malarial parasite caused a decrease in levels of fatty acids 16:0 and 18:0 but increased 18:2 and 20:4. These effects resemble those in *Table 13*, reported for effects of temperature on female rat serum fatty acids, particularly in the

Table 13. Effect of temperature on unsaturation of female rat serum fatty acids

	mol %		mol %
Fatty acids	15°C	30°C	15°C − 30°C
16:0	18·3	23·6	−5·3
16:1	1·9	2·9	−1·0
18:0	13·8	13·7	+0·1
18:1	11·3	15·8	−4·5
18:2	26·1	25·9	+0·2
20:3	1·0	0·8	+0·2
20:4	24·1	15·0	+9·1
22:5	1·1	0·7	+0·4
22:6	2·4	1·6	+0·8

reduction of 16:0 and increase of 20:4. It should be noted that the study by Stocker *et al.* (1987) used male mice whereas the work by Gonzalez, Nervi and Peluffo (1986) was with female rats. The differences in experimental stresses, sex and species are considerable and so the similarity of physiological responses in fatty acid unsaturation is quite remarkable.

In discussing the surface waxes of insects, Hadley (1980) has noted that experiments performed on artificial bilayers and plasma membranes have shown that water permeability was decreased by long-chain saturated molecules and increased by unsaturated short-chain and branched-chain structures. In terms of *n*-alkanes, long-chain saturated compounds should afford maximum waterproofing for insects subjected to high temperatures, while unsaturated and branched-chain alkanes should be more subject to water loss. In addition, long-chain saturated compounds, because of their higher melting points, should give increased temperature stability compared with unsaturated and branched-chain molecules. In fact, *Elodes armata* beetles, active in the summer or in the winter but acclimatized to summer temperatures, had a higher level of long-chain saturated alkanes in their cuticles than did winter-active or control beetles. In the scorpion, *Centruroides sculpturatus*, summer epicuticular hydrocarbons were predominantly long-chain branched alkanes whereas scorpions studied in the fall and winter provided more short-chain alkanes. The response in the lipids of the animal kingdom towards decreased temperature favours increased unsaturation, branching and short chains, as with the plants and micro-organisms.

Lipids and enzyme activity

The preceding discussion has indicated the effects of temperature and other environmental factors on lipid structural elements, including unsaturation, chain length, branching, hydroxylation and cyclization as well as changes in the relative concentrations and locations of lipid classes such as phospholipids. These changes in lipid structure and distribution mediate alterations in membrane properties such as fluidity, nutrient transport and enzyme activity. The chemical and physical properties of lipids may influence enzyme activity in several ways through effects on substrate and product solubility, enzyme conformation and enzyme induction (Sandermann, 1978; Cunningham and Sinthusek, 1979; Gabrielides, Hamill and Scott, 1982; Brenner, 1984). Among the lipid properties that may be involved in affecting enzyme reactions are rigidity, thickness of lipid layer, relative hydrophobicity/hydrophilicity, hydration and surface charge.

The enzymes affected by changes in lipid structure and distribution can be divided into two arbitrary groups: the first group includes those directly involved in lipid synthesis—those, for example, involved in desaturation, chain-length determination and phospholipid formation; the second group includes all other enzymes—those not directly involved in lipid synthesis, but rather in the myriad of other reactions intrinsic to life processes.

Numerous enzymes that are lipid-dependent or lipid-modulated have been reported (Gennis and Strominger, 1976; Sandermann, 1978; Caffrey and

Feigenson, 1981; Johannsson *et al.*, 1981; Gabrielides, Hamill and Scott, 1982; Stadtlander, Rade and Ahlers, 1982; Brenner, 1984; Kates, Pugh and Ferrante, 1984; Ayala *et al.*, 1986; Froud *et al.*, 1986; Laird *et al.*, 1986). Some of these are listed in *Table 14*.

The mechanisms by which temperature affects lipid unsaturation have been discussed in several reviews (Herbert, 1981; Cossins, 1983; Thompson, 1983; Brenner, 1984; Russell, 1984; Kasai *et al.*, 1985; Thompson, 1985). The biochemical pathways for fatty acid desaturation are known to a great extent, but the regulation of these biosynthetic steps is still largely speculative.

In eukaryotic cells, such as *Tetrahymena* and mammalian cells, three types of regulatory mechanisms have been suggested: (1) low-temperature-induced synthesis of new desaturase molecules; (2) modulation by membrane fluidity of existing desaturase molecules; and (3) inhibition of desaturases by polyenoic fatty acids. In prokaryotic cells, two major regulatory mechanisms have been presented. In *Escherichia coli* a temperature-labile enzyme, β-ketoacyl-ACP-synthase II (3-oxoacyl-[acyl-carrier-protein] synthase; EC 2.3.1.41), specifically increases the rate of unsaturated fatty acid synthesis at low temperatures by elongating palmitoleic acid (16:1) to *cis*-vaccenic acid (18:1), which is then incorporated into unsaturated phospholipids. A different regulatory mechanism exists in *Bacillus megatherium*: in this case, there is desaturase induction mediated by a temperature-sensitive modulator. The modulator is temperature-labile at 35°C and is, therefore, most active at low temperatures. There is also a second mechanism in this micro-organism: the desaturase enzyme is high-temperature-labile and has a lower turnover rate at low temperatures; thus, when growth temperature is lowered, more enzyme activity is available until it gradually declines in adjustment to the new growth conditions. This process provides the cell with a quick response device, using pre-existing desaturase molecules. In instances such as this, there is certainly a reciprocal effect in that enzyme protein modifies lipid structure and then the modified lipid structure alters enzyme protein conformation. In some cases, the protein may be that of a single enzyme, as in the case of the desaturase involved in aspects of lipid synthesis. In other instances, more than one enzyme protein may be involved as, for example, when the first is concerned with modified lipid production (the desaturase) and the second is not involved in lipid synthesis at all, but merely responds to the modified lipid, such as enzymes indicated in *Table 14*.

The relationship between enzyme activity and lipid structure and distribution as a function of temperature might be clarified by studies using purified enzymes and lipids over a range of temperatures. It is to be anticipated that the compounds showing optimal activation would differ as the temperature changes. Most of the studies reported in the literature have been carried out at a single temperature, usually either 25°C or 37°C.

Bruni, Van Dijck and DeGier, (1975) showed that the activity of bovine heart mitochondrial ATPase (EC 3.6.1.34) was regulated by the chain length and unsaturation of phospholipids. Gennis and Strominger (1976) indicated that the branched-chain fatty acid 12-methyltetradecanoic acid and the

Table 14. Enzymes dependent on or activated by lipids

Enzyme	Source	Lipid activators	References
C_{55}-isoprenoid alcohol phosphokinase (undecaprenol kinase; EC 2.7.1.66)	*Staphylococcus aureus*	Phospholipids, fatty acids, detergents	Gennis and Strominger, 1976
$(Ca^{2+} - Mg^{2+})$ – ATPase H^+-transporting ATP synthase; EC 3.6.1.34)	Rabbit sarcoplasmic reticulum	Phospholipids, fatty acids	Froud *et al.*, 1986
Δ^9-(Steroyl-CoA) and Δ^{12}-(oleoyl-CoA) desaturases (acyl-CoA desaturase; EC 1.14.99.5)	*Neurospora crassa*	Stearic and oleic acids, anionic phospholipids	Gabrielides, Hamil and Scott, 1982
Protein kinase (C-kinase) (EC 2.7.1.37)	Mammals, mollusc *Hermissenda crassicornis*	Diacylglycerol, phospholipids	Neary *et al.*, 1986
$F^0 \cdot F_1$ – ATPase (H^+-transporting ATP synthase; EC 3.6.1.34)	Beef heart mitochondria	Phospholipids	Laird *et al.*, 1986
3-Hydroxybutyrate dehydrogenase (EC 1.1.1.30)	Beef heart mitochondria	Phosphatidylcholine	Sandermann, 1978
Acetylcholinesterase (EC 3.1.1.7)	Erythrocytes	Cardiolipin	Sandermann, 1978
Epoxide hydrolase (EC 3.3.2.3)	Liver	Phosphatidylcholine	Sandermann, 1978

unsaturated *cis*-oleic and vaccenic acids were superior to straight-chain saturated and *trans*-unsaturated fatty acids for the activation of C_{55}-isoprenoid alcohol phosphokinase (EC 2.7.1.66) from *Staphylococcus aureus*. Caffrey and Feigenson (1981) reported that the ATPase of rabbit sarcoplasmic reticulum (EC 3.6.1.34) was sensitive to the acyl chain length in phosphatidyl choline analogues, with little sensitivity to the details of unsaturation. These three studies were carried out at 25°C. Froud *et al.* (1986) in studying the latter enzyme at 37°C showed that 18:1 phosphatidyl choline yielded optimal activity in a study of a series of analogues ranging from 14:1 to 24:1. Studies such as these and others in the recent literature (Johannsson *et al.*, 1981; Laird *et al.*, 1986; Neary *et al.*, 1986) were designed to afford an understanding of lipid–enzyme interactions at a given temperature but do not significantly broach the relationship of lipids and enzymes over a temperature range. If, in each case, a parallel study had been done at 15°C, more basic information relevant to the interplay of lipid structure and properties on enzyme activity as a function of temperature would have been revealed. The data obtained from such experiments *in vitro* may have only limited value

in understanding interactions *in vivo*, because of the absence of membrane complexity and integrity, but such information may contribute to more effective utilization of enzymes in organic solvents for commercial applications. Nature and industry are not necessarily trying to solve the same problems.

In this respect, a growing area of experimentation in biocatalysis is that concerned with enzyme reactions under low water and high organic solvent conditions (Klibanov, 1986). Among the effects under study are those involving enzyme conformation, hydration and stability, the reversal of several hydrolytic processes to synthetic reactions, and substrate solubility and availability. Enzymes naturally located in areas of high lipid content are doubtless subjected to some of the same environmental pressures *in vivo* as are enzymes in organic-rich *in vitro* reactions.

Sandermann (1978) and Cossins (1983) have noted that a number of enzyme systems, including the succinate dehydrogenase (EC 1.3.99.1) of the epoxial muscle of the goldfish, have a lipid cofactor requirement when reacting with water-insoluble substrates. As the substrate solubility in lipid will vary as a function of temperature, one would expect that the optimal lipid structure would also change with temperature. Does this factor operate as one consideration in determining the requisite modification in membrane lipids under temperature stress? In the case of the succinate dehydrogenase, Hazel (1972) showed that total mitochondrial lipid from goldfish (*Carassius auratus*) acclimatized to 5°C was more effective in restoring activity to delipidated enzyme than lipid from goldfish acclimatized to 25°C. The magnitude of reactivation depended largely on the unsaturation of phospholipid acyl chains. Was this effect on substrate solubility, enzyme conformation, or both?

Conclusions

The reading of this review may result in the reader having more unanswered (but different) questions than previously. That is because much of the biochemistry and biophysics of the relationship between temperature and lipid unsaturation and structure, in general, remains to be clarified by future research. For the sake of simplicity and the importance of carrying away at least a single message, let it be that there is, in many instances, an inverse relationship between temperature and unsaturation. To produce unsaturated chemicals, the biosynthetic steps should be carried out at the lowest temperature compatible with reasonable economics and technology. To produce more temperature-hardy crops, high unsaturation is a good assumption for chill resistance and low unsaturation is likewise for heat resistance.

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