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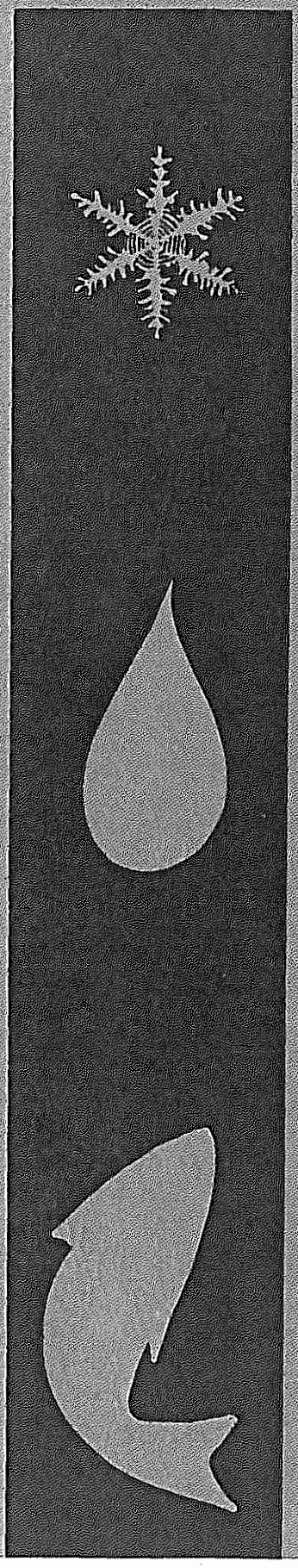

The Biochemical Bases of Psychrophily
in Microorganisms

A Review
by
Ann P. Miller

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PREFACE

As a biochemist involved in problems of water pollution and waste treatment in Alaska one would logically be concerned about the metabolic capabilities of the microorganisms indigenous to the arctic and sub-arctic environments. In our laboratory we are interested in the ability of natural waters to assimilate and "purify" wastes emptied into them during the winter months as well as the ability of biological waste treatment units to operate outdoors at a time of year when the air temperature can easily drop to -40°C and lower. The extent to which biological purification of wastes can take place under such conditions is a function of the microorganisms involved, their metabolic rates and abilities to grow at low temperatures.

The review which follows was undertaken to ascertain just what is known about microorganisms indigenous to cold environments, for these are the ones we can expect to be most important in our work. It was found that research on the true "cold-loving" microorganism, the psychrophile, is still in the initial stage, where for every question asked, one receives a half-dozen more in reply. Nevertheless, it is hoped that the information presented in this paper will be of benefit to others involved in environmental health-related research at northern latitudes, if in no other way than by assembling in one place what is known about

psychrophiles and by describing the limitations of our present knowledge of such microorganisms.

I wish to express my thanks to Dr. R. Sage Murphy for his suggestions and to the Institute of Water Resources with whose support this review was made.

A. P. M.

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INTRODUCTION

Microorganisms capable of growth even at subzero temperatures have long been known; however, most have consistently fared better at higher temperatures, usually above 20°C. Much of the work done with the biological oxidation of wastes at low temperatures has been with organisms of this type: mesophilic organisms which are able to survive at low temperatures but which are physiologically much more active in the range from 20°C to 45°C. Such organisms might be labeled "cold-tolerant," but they are probably biochemically quite different from the truly "cold-loving," or psychrophilic, microorganisms which are able not only to survive but also to thrive at temperatures below 20°C and which, in fact, find temperatures much higher than 25°C intolerable.

Until recently the psychrophilic microorganism was more a hypothetical beast than an established fact. A strict definition of a psychrophile is an organism which grows rapidly enough at 0°C on solid media so as to be visibly detectable within a week and whose optimum growth temperature is 20°C or lower.[33] Psychrophiles differ from mesophiles in being able to grow at much lower temperatures and in being capable of greater physiological activity than the mesophiles in the region where the temperature ranges for growth of the two groups overlap. Microbiologists were slow to discover an organism which fit the

definition. The marine environment, with its characteristic temperatures of 5°C and lower, seemed a logical place to look, and indeed Morita[31] reports that numerous obligate psychrophilic bacteria have been isolated from the sea. One example, *Vibrio marinus* MP-1, was shown to have an optimum growth temperature of 15-16°C and a maximum temperature for growth of less than 20°C. This bacterium was able to grow at -1°C but its viability was adversely affected by temperatures between 20°C and 30°C.[36] Other obligate psychrophiles have now been isolated,[11,33] but because these few isolates have been discovered only recently, much of what follows describes experiments with organisms which do not fit precisely the definition given above in that their optimum growth temperatures are above 20°C. It must be added that as yet no obligate psychrophiles have been isolated from a freshwater environment, but this is most likely because they have not been actively sought there.

Once its existence had been confirmed, the psychrophile became the object of active inquiry, for the psychrophile becomes an especially interesting organism when one realizes that microorganisms have no mechanism for conserving heat or retarding its dissipation and that consequently they lose their metabolic heat easily to their aqueous surroundings. How then, one asks, are they able to maintain levels of activity, at low temperatures, which are comparable to those of mesophiles at higher temperatures? Their adaptation to cold environments must involve some decidedly different biochemical arrangements within the cell. Determining just what these different arrangements are is the goal

of present-day investigators, for these mechanisms are the key to the distinguishing character of the psychrophile.

THE BIOCHEMICAL BASES FOR THE LOW MINIMUM GROWTH TEMPERATURES

The temperature coefficient. Early investigators of the effects of temperature on biological processes considered each physiological activity to be but a series of chemical reactions, albeit complicated, and therefore subject to the same laws as chemical reactions. According to this reasoning, one would expect that for each 10°C rise in the temperature there would be an increase in the rate at which the activity occurred, so that the process would occur approximately 2 to 3 times faster at the higher temperature, as is usually found for thermochemical reactions.[15] The "temperature coefficient," or Q_{10} value, is used to describe this ratio of the reaction velocity at one temperature, K_t, to that at another temperature 10°C lower, K_{t-10}:

$$Q_{10} = \frac{K_t}{K_{t-10}}.$$

Whereas such a relationship usually holds true for chemical reactions, it does not apply to the process of growth and to other biochemical activities, whose Q_{10} values vary with the temperature range and the organism. Moreover, these values have repeatedly been found to be higher in the lower temperature range than would be expected for simple chemical reactions. (Table I.)

Table 1. Temperature coefficients (Q_{10} values) of growth at various temperatures[9]

Organism	Temperature Range, °C						
	-10 to 0	-5 to 5	0 to 10	5 to 15	10 to 20	15 to 25	20 to 30
Aerobacter aerogenes			9.1		3.1		1.6
Pseudomonas 92			4.8		3.2		1.8
Pseudomonas 69			4.4		3.4		2.5
Thamnidium chaetocladiodes		16.6	3.8	1.9	1.4		
Cladosporium herbarum		10.7		4.1			
Thamnidium elegans		132.0		2.2			
Sporotrichum carnis		7.2		2.4		2.4	
Pseudomonas fluorescens	9.3		8.4	3.7			
Streptococcus fecalis			{7.0 8.6				2.4
Achromobacter sp.		5.3		2.3		2.3	

The Arrhenius relationship and the "master reaction." The effect of temperature on growth and other biochemical activities of the cell has been considered also from the standpoint of the Arrhenius relationship:

$$\ln K = -\mu/RT + \text{constant},$$

where K is the reaction velocity at absolute temperature T, R is the universal gas constant, and μ is another constant, commonly called the "temperature characteristic" of the reaction. The temperature characteristic is also the apparent activation energy, in calories per mole, for the reaction. This value can be determined from the slope of the curve obtained by plotting the logarithm of the growth or activity rate against the reciprocal of the absolute temperature. (Figure 1.)

Since μ was found to be fairly constant over a limited temperature range, it was construed by some to reflect the apparent activation energy of one reaction in particular; namely, a "master reaction" which dominated the process' overall response to temperature change. Slight breaks in the linearity of Arrhenius plots were interpreted by Crozier[8] and others as corresponding to "critical temperatures" at which a new "master reaction" became important. Thus, at high temperatures the reaction which determined the rate of the whole process would be one with a low energy of activation, whereas at low temperatures the limiting reaction would be one with a relatively high energy of activation. It was hoped that it would be possible to identify the particular enzyme system responsible for the observed temperature response of a given

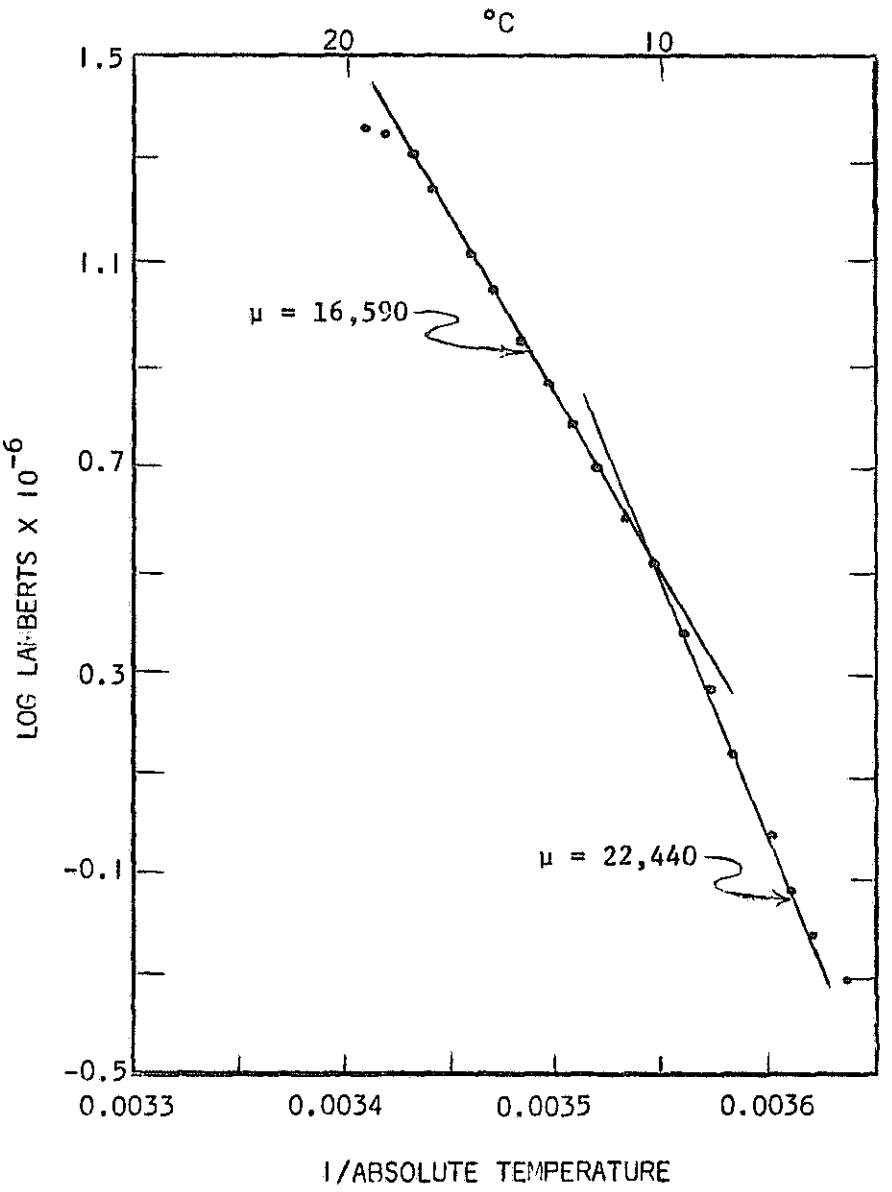


Figure 1. Effect of temperature on intensity of light from luminous bacteria.[9]

physiological activity by comparing the apparent activation energy for the process with activation energies for various enzyme reactions. This hope has been largely abandoned, for, although the reactions which take place within the cell are indeed chemical reactions and as such are subject to physical and chemical laws, it is now realized that a process such as growth is highly complex and is not likely to consist simply of a series of reactions dominated by a single controlling reaction. Moreover, the "breaks" in Arrhenius plots have since been shown to be of doubtful authenticity.[20] Deviations from the Arrhenius straight line are actually gradual and are better represented by slightly curved plots. These deviations from linearity have also been interpreted as resulting from a reversible inactivation of enzymes at low temperatures.[23,27] According to this theory, the inactive form of the enzyme is one in which the protein moiety is rigid and insufficiently unfolded because of a greater degree of intramolecular hydrogen bonding and it is thus unable to perform its function as a catalyst.

Energies of activation for cellular processes. The concept of the temperature characteristic, μ, is relevant nevertheless to an understanding of why a microorganism has a minimum temperature below which it cannot grow and why this minimum varies from one microorganism to the next. Changes in μ for various cellular activities parallel the changes in Q_{10} with temperature; the energies of activation may be found to increase as the temperature is lowered, becoming quite large near the minimum growth temperature of the organism.[9,26] For example, Sultzer[48]

noted that as the temperature was lowered, the value of μ for the oxidation of saturated fatty acids showed an increase at about 15°C for two psychrophilic pseudomonads. A corresponding increase in the energy of activation for the oxidation process was noted with the two mesophiles, *Serratia marcescens* and *Sarcina flava*, but at about 20-25°C. Moreover, the psychrophilic microorganisms oxidized almost all of the fatty acids tested more rapidly than the mesophiles at all temperatures tested from 7.5°C to 37.5°C and displayed lower energies of activation for the process. (Table 2.) The oxidative activity of the psychrophiles was seen to be less sensitive to a decrease in temperature than was that of the mesophiles, possible indicating that the two types possess different enzyme systems and/or different reaction pathways for the same process.

Table 2. Temperature characteristic (μ) values for the oxidation of sodium octanoate[48]

Organism	μ cal/mole	Temperature range °C
Pseudomonas sp. I	7,630	15 - 37.5
	16,030	7.5 - 15
Pseudomonas geniculata	6,870	15 - 35
	14,660	7.5 - 15
Sarcina flava	9,160	22 - 37.5
	22,900	15 - 25
Serratia marcescens	9,160	20 - 35
	18,320	7.5 - 20

Ingraham[17] compared the Arrhenius curves for growth of a facultative psychrophilic pseudomonad and a mesophile, *Escherichia coli* K-12. (Figure 2.) The shapes of the curves were similar; the important difference lay in the slopes in the linear portion. These different slopes correspond to a temperature characteristic for growth of about 14,000 calories per mole for the mesophile and only about 9,000 calories per mole for the psychrophile. Ingraham sought to explain this difference by making a comparison of the effects of temperature on enzymes obtained from both psychrophiles and mesophiles. He studied three enzyme pairs: malic dehydrogenase, isocitric dehydrogenase, and glucose-6-phosphate dehydrogenase. However, the apparent activation energies were essentially the same for mesophilic and psychrophilic enzymes. The enzymes from the psychrophilic pseudomonad did not have lower activation energies than those from the mesophiles as was expected. Ingraham abandoned this approach on the basis that the likelihood of finding the critical enzyme pair, if there were one, was too remote. He thought rather that the difference in response of the two organisms to various temperatures was related somehow to the intact cell. He therefore compared psychrophiles and mesophiles with respect to the effect of temperature on glucose oxidation by whole cells.[17] These experiments revealed that the temperature coefficients, (Q_{10} values), for glucose oxidation were much less for the psychrophiles. Ingraham concluded that "the temperature response differences between psychrophiles and mesophiles for growth and catabolism are probably the result of some aspect of cellular organization rather than of enzymic differences."

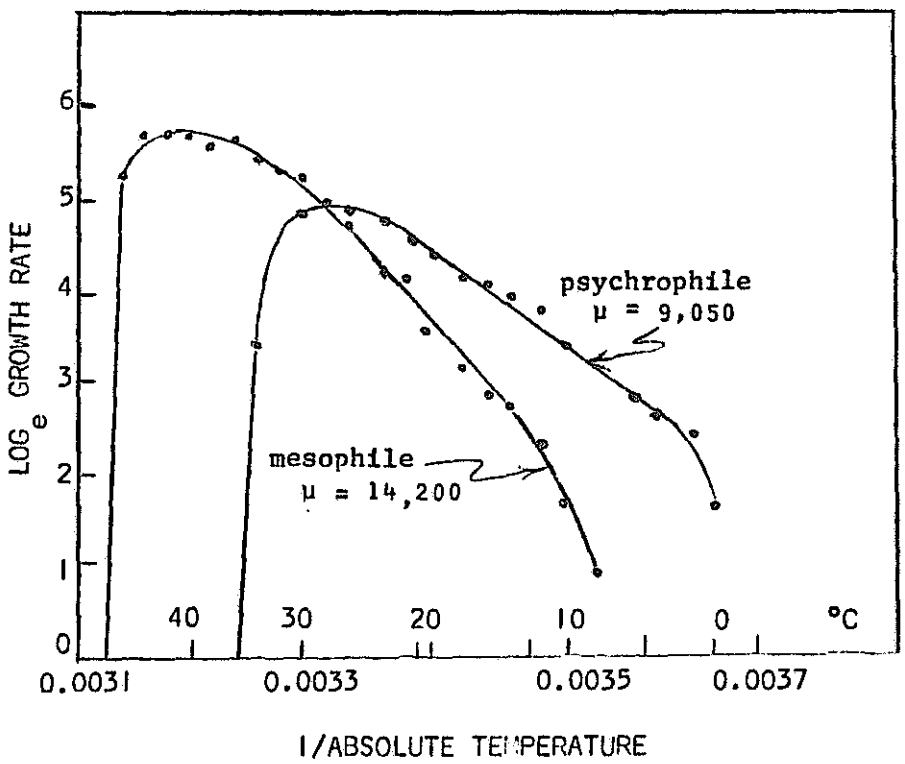


Figure 2. Arrhenius plot of growth rate of a psychrophilic pseudomonad as compared with that of a mesophile.[17]

On the other hand, Burton and Morita[5] more recently reported that the energy of activation of malic dehydrogenase from *Vibrio marinus*, an obligate psychrophile, was found to be one-half the energy of activation of the same enzyme from *E. coli*, and further that the energy of activation for the synthesis of galactosidase in obligate psychrophiles inducible for this enzyme was significantly lower than that in *E. coli*. These facts lend support to the hypothesis that an overall lower energy of activation for cellular reactions contributes to psychrophilic growth.

Permeability, active transport, and fatty acids. Baxter and Gibbons[2] compared the temperature response of respiration in a strictly psychrophilic species of *Candida* with that in a mesophilic species, *Candida lipolytica*. The psychrophile exhibited greater endogenous and glucose respiratory activities at low temperatures (0°C to 30°C) than did the mesophile, but at 40°C its respiratory activity abruptly ceased, apparently due to the inactivation of one or more of the enzymes involved in respiration. On the other hand, these activities increased in the mesophile with increasing temperature and were greater at 40°C than at the lower temperatures. (Figure 3.) Why did these two related species respond so differently to the same temperatures? Possibly the observed difference in respiratory activity was due to different enzymic mechanisms, or perhaps it was due to differences in the permeability of the cell membranes to glucose. That is, the reduced respiratory activity of the mesophile at low temperatures might be a reflection of its inability to take glucose into the cell in the first place.

To determine whether this latter hypothesis was correct, the permeability of each yeast to sugars was evaluated by measuring the rate at which glucosamine was accumulated within the cells at various temperatures. The results are shown in Figure 4. The rate of glucosamine uptake in the psychrophile was only slightly temperature dependent between 0°C and 30°C, while in the mesophile virtually no uptake occurred at 0°C, and at 20°C the rate was less than a third that at 30°C, where it was maximum.

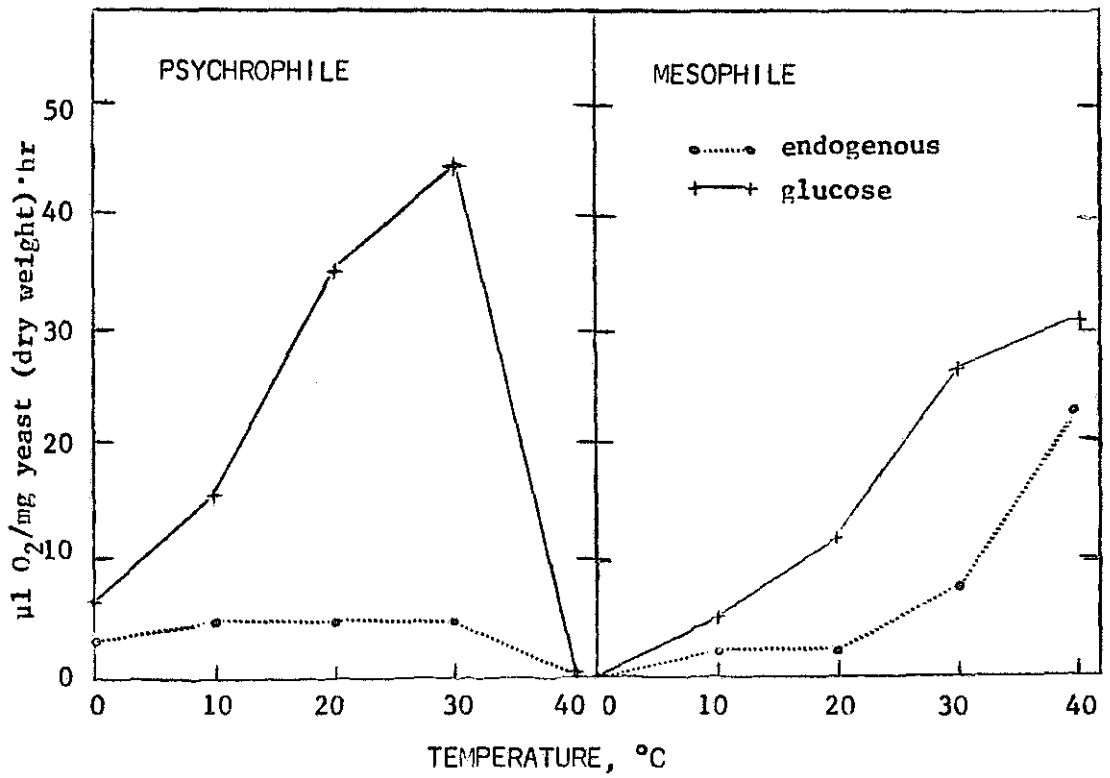


Figure 3. Influence of temperature on oxidation of glucose by psychrophilic and mesophilic species of *Candida*.[2]

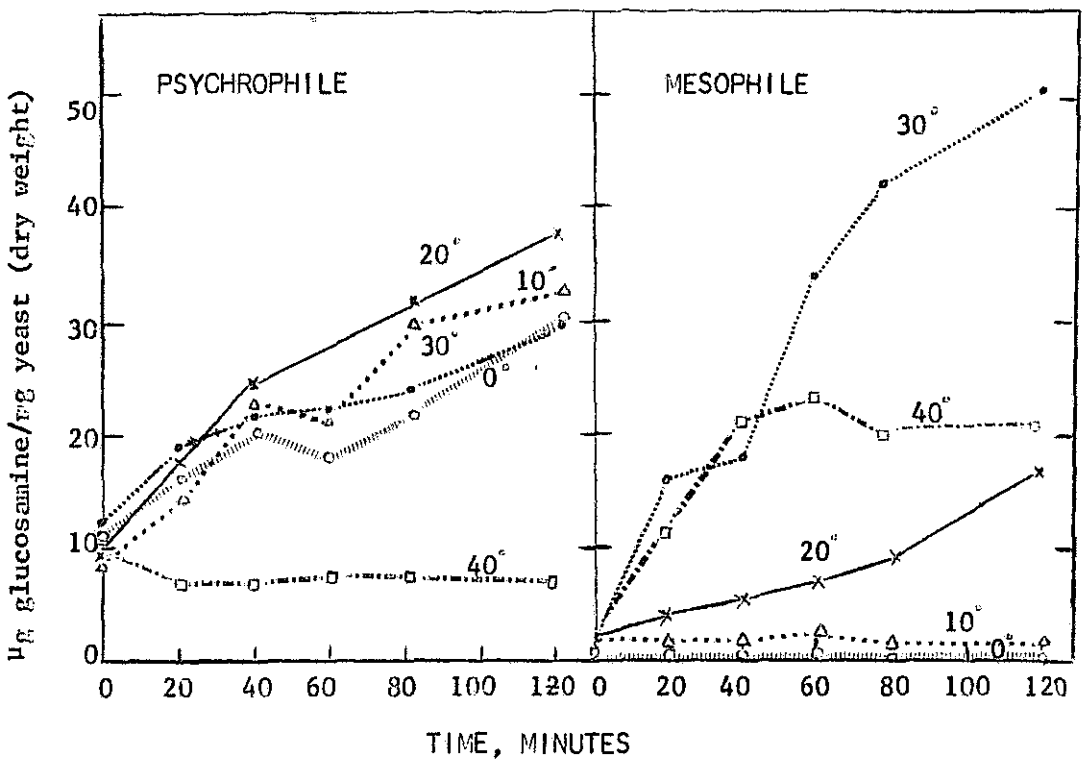
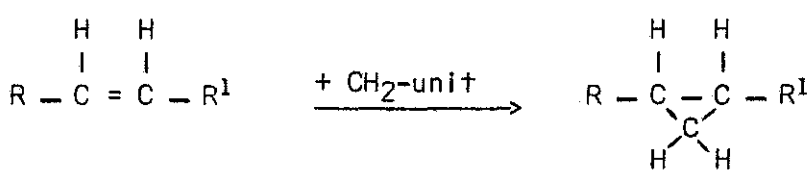


Figure 4. Glucosamine uptake by psychrophilic *Candida* No. 5 compared with that by *Candida lipolytica*, a mesophile.[2]

Because of the strikingly different permeabilities of the mesophile and the psychrophile at low temperatures, Baxter and Gibbons speculated that the site of inhibition of respiration by cold in the mesophile lay in one or more of the cell's transport mechanisms. More specifically they suggested that the mesophile's reduced permeability at low temperatures might be due to a closer packing of the lipid molecules in the cell membrane as compared with that of the psychrophile. This suggestion was based on the finding of Kates and Baxter[21] that the fatty acids of this same psychrophile species of *Candida* grown at 10°C are more highly unsaturated than those of the mesophilic species grown at 25°C, (1.4 to 1.7 double bonds per mole as compared with 1.0 per mole). Not unexpectedly, growth at elevated temperatures results in more saturated fatty acids in the lipids of various organisms than are found at normal temperatures. This result has been interpreted in terms of the need for maintaining a protoplasmic viscosity suitable for cellular activities,[1] saturated fatty acids being less "fluid" than their unsaturated counterparts. In like manner, a greater proportion of unsaturated fatty acids in the lipids might be diagnosed as resulting from the need for maintaining protoplasmic viscosity at low temperatures, for the "liquid character" of a hydrocarbon chain is related to its degree of unsaturation, and when water is present this "liquid character" may be important in determining the phase which can exist at any particular temperature.[6] These facts, coupled with the indication by Hokin and Hokin[16] that the

turnover of a phospholipid (phosphatidic acid) in the avian salt gland may function in sodium transport, suggest that the degree of unsaturation of lipids may be related to permeability processes.

Kates and Hagen[22] examined the fatty acid composition of a psychrophile, believed to be a species of *Serratia*, and of a mesophile, *Serratia marcescens*, when both were grown at 10°C. The fatty acids of the psychrophile were found to contain high proportions of unsaturated acids. When the mesophile was grown at 10°C, the degree of unsaturation of its fatty acids approached that of the psychrophile. At this temperature the mesophile contained more monoenoic acids than it did when grown at 30°C. At 30°C the mesophile produced large amounts of cyclopropane acids, and although at 10°C it still synthesized appreciable quantities of these acids, the psychrophile synthesized none. Yet both organisms contained phosphatidyl ethanolamine. The provision of methionine and S-adenosyl methionine, known to be necessary for the biosynthesis of cyclopropane acids, did not affect the fatty acid composition of the psychrophile with respect to these acids. Kates and Hagen thought it difficult to avoid the conclusion that the psychrophile lacked the enzyme which catalyzes the transfer of a methylene group to the double bond of the monoenoic acids present in the phosphatidyl ethanolamine:



They suggest that this loss might have occurred as a result of adaptation to growth at low temperatures, because this enzymatic reaction may represent a mechanism for protecting the olefinic compound in the cell membrane from peroxidation at normal temperatures, a mechanism which may not be necessary at lower temperatures.[25]

Proteins and transport mechanisms. Rose and Evison[41] also have attempted to assess the extent to which temperature characteristics of the solute transport mechanisms determine the minimum temperatures for growth in microorganisms. Their experiments to determine the effect of temperature on respiration and glucosamine uptake in several psychrophiles and mesophiles were similar to those of Baxter and Gibbons.[2] Respiration of exogenous glucose at 0°C was detectable in each of the psychrophiles studied; however, with the three mesophiles respiration was negligible below 5°C. In addition, the ability of each organism to accumulate glucosamine between 0°C and 25°C was affected in the same way as its ability to respire glucose. All of the psychrophiles were able to transport sugars across the cell membrane even at 0°C. Apparently the inability of the mesophilic microorganisms to respire glucose below 5°C was the result of their not being able to transport sugars into the cell at such temperatures, because when one of the mesophiles, *Candida utilis*, was treated with 2% (v/v) aqueous butanol to break down the osmotic barriers and permit free diffusion of solute into the cells, it was then able to respire glucose below 5°C. Rose and Evison[41] concluded that the minimum growth temperatures of mesophiles may be determined, at least

partly, by the inactivation of the mechanisms for transporting sugars into the organisms at low temperatures. They state that there is little evidence for the participation of triglycerides or phospholipids in transport processes in microorganisms and, unlike Baxter and Gibbons, think it more likely that the carrier molecules are mainly protein in nature and undergo conformational changes in effecting the transport of solutes. They suggest that in mesophiles the protein carriers become hyperfolded at low temperatures and are thus unable to combine with the solute. But if the carrier molecules are primarily protein in the mesophile, they should logically be so in the psychrophile as well; yet Rose and Evison offer no suggestion as to how the psychrophile might have overcome this problem. And in fact, for three strains of the mesophile *Corynebacterium xerosis* which they studied, the minimum temperatures for growth were definitely not determined by the temperature at which sugar transport processes were inactivated.

Loss of control of enzyme synthesis. Another type of cellular alteration which has been suggested to account for the different responses to temperature by psychrophiles and mesophiles is loss of control over enzyme synthesis in the mesophile exposed to low temperatures.[17,30,37] Ng, Ingraham, and Marr[37] reported evidence of damage to cells of *E. coli* by growth at temperatures below 20°C which resulted in a lowered specific growth rate. This "damage" was a progressive decrease in the glucose repression of the synthesis of β-galactosidase as the growth temperature decreased and eventually the complete release of repression at 10°C. The

damage was not irreparable, however, and could be corrected by a period of growth at a higher temperature. Nevertheless, the loss of control over certain repressible and inducible enzymes might result in the increase in the temperature characteristic for growth at low temperatures previously noted for *E. coli*. (Figure 2.) That is to say, energy which at higher temperatures and under conditions of regulation of enzyme synthesis would be utilized for growth and reproduction might at lower temperatures, where control is lost, be shunted off in ineffectual directions, thus increasing the overall energy requirement for growth. Yet the question remains: Does the psychrophile have to cope with a similar lack of control? If so, how is it able to overcome it? If not, how does it manage to avoid it?

THE BIOCHEMICAL BASES FOR THE LOW MAXIMUM GROWTH TEMPERATURES

The foregoing discussion has been concerned primarily with the question: What enables certain microorganisms to grow at very low temperatures? What follows will be aimed particularly at discovering what determines the relatively low temperatures above which these same microorganisms cannot grow. These two problems need not be one and the same.

Increased nutritional demands. In a comparative study of the biochemical bases of the maximum temperatures for growth of three psychrophilic microorganisms, Evison and Rose[10] transferred cultures of these organisms, which were growing at or near their optimum growth temperatures, to temperatures 3-5°C above their *maximum* growth temperatures. After being returned to their optimum temperatures, cultures of *Candida* and *Arthrobacter* showed a lag before growth was resumed, and this lag period was a function of the length of time spent at the higher temperatures. *Corynebacterium erythrogenes*, on the other hand, grew almost immediately upon being returned to its optimum. Evison and Rose considered that perhaps at the higher temperatures the organisms required growth factors which had not been required at the optimum temperatures and which were not available in the media provided. They supplemented the media with complex nutrients such as yeast extract and bacteriological

peptone but with no detectable effect on growth at the higher temperatures. Thus, it appeared that low maximum growth temperatures are an intrinsic characteristic of psychrophiles and not due to increased nutritional demands.

Denaturation of nucleic acids. It has been suggested that the damaging effect of high temperatures on microorganisms is caused, partly at least, by the breakdown of the nucleic acids. However, Evison and Rose[10] found little difference in the amounts of ultraviolet-absorbing compounds in cells of their cultures before and after transfer to temperatures above maximum. Nor does it seem likely that degradation of the nucleic acids would be important in determining the maximum temperatures of growth for psychrophiles. With natural deoxyribonucleic acids (DNAs), at least, the transition from the helical configuration to a disordered strand takes place within the narrow temperature range from 80°C to 100°C,[28] temperatures much too high for this type of degradation to be considered applicable to psychrophiles. The minimum temperature at which this "melting" of a DNA helix occurs was seen to be about 65°C, and this was in the case of an artificially prepared DNA consisting solely of adenine-thymine base pairs. Inclusion of guanine-cytosine base pairs in the structure only tends to raise the melting point.[28] Even though certain organic solvents, purines and pyrimidines, electrolytes, and physical agents tend to lower the thermal denaturation temperature of DNA,[29] the effect is probably not great enough to cause denaturation at 20°C to 30°C. However, thermal denaturation of ribonucleic acids (RNAs) occurs

at lower temperatures and, as is the case for DNA, bears a linear relationship to the amount of guanine-plus-cytosine in the molecule. The mean denaturation temperatures of several RNAs which have been tested, varying in guanine-cytosine content from 44% to 61%, are in the range of 48°C to 60°C. This lower range is a reflection of the structure of the RNA macromolecule; it is not a rigid double helix like DNA but a flexible, single-stranded polyribonucleotide chain which coils into compact particles in solution.[46] RNA has no fixed secondary structure but by forming intramolecular hydrogen bonds takes up the most stable conformation possible under the dictates of its nucleotide sequence and the surrounding medium. Therefore, since the secondary structure of RNA changes with surrounding conditions and because the thermal unfolding of the chain is completely and instantly reversible,[46] it appears that heat denaturation of RNA does not truly occur in the sense that the molecule is irreversibly damaged at moderate temperatures. However, the possibility remains that prolonged exposure to elevated temperatures might interfere with the functions of RNA and that extreme heat treatment might cause disintegration of the macromolecule into smaller units.

Exceptionally heat-sensitive enzymes. In the experiments of Evison and Rose,[10] transfer of *Arthrobacter* and *Candida* cultures to higher temperatures had caused a rapid decline in their rates of respiration of exogenous glucose and endogenous reserves, accompanied by a marked decrease in viability. Thermal stresses might possibly have caused lesions in the respiratory pathways of these organisms. Indeed, it was seen that

the transfer to the higher temperatures had caused a definite reduction in the activities of some of their tricarboxylic acid cycle enzymes, especially isocitric dehydrogenase. Thus, it would appear that exceptionally heat-labile respiratory enzymes are responsible for the low maximum growth temperatures of these psychrophilic microorganisms.

Hagen and Rose[13] came to the same conclusion regarding a psychrophilic species of *Cryptococcus*. This organism was induced to grow rapidly for a short time at a temperature above its maximum growth temperature by previous incubation at 16°C, a temperature at which it normally grew well. No significant difference was found in the nucleic acid or protein content of the cells before and after transfer to the new temperature of 30°C. However, major differences were observed in the content of intracellular amino acids, there being a rapid utilization of intracellular reserves of these acids upon transfer to 30°C. In particular, there was a rapid decline in the amount of glutamic acid in the amino acid pool, and cessation of cell division coincided with exhaustion of this reserve. When the *Cryptococcus* was returned to 16°C, growth began again after a lag period and the amino acid pool was once again replenished. Yet supplementing the medium with peptone, yeast extract, and DL-glutamic acid had no effect on growth at 30°C. These observations suggested that one reason for the failure of the *Cryptococcus* to continue to multiply at 30°C was its inability both to synthesize amino acids and to accumulate them from the medium.

Since certain amino acids are known to be synthesized from α-keto

acids, (e.g., glutamic acid from α-ketoglutaric acid), Hagen and Rose[13] studied the effect of transfer to the higher temperature on the total keto acid content of the *Cryptococcus*. The data were similar to those for intracellular amino acids, describing a steady decline in the total amounts of these acids from cultures that had been transferred from 16°C to 30°C, and suggesting that at 30°C the *Cryptococcus* is unable to synthesize adequate amounts of keto acids, α-ketoglutaric acid in particular. It would not be surprising if such an impairment were to be accompanied by deterioration of the organism's respiratory metabolism.

Since the *Cryptococcus* is apparently unable to take in appreciable quantities of exogenous amino acids at 30°C, even when the organism is deficient in these compounds, it may be incapable of furnishing the energy required for the uptake of these nutrients at this temperature. It appears that this inability is due to certain exceptionally thermolabile enzymes. The fact that the organism can survive at 30°C and is capable of synthesizing and accumulating amino acids within the cell upon its return to 16°C indicates that the heat inactivation of these enzymes is reversible at 30°C. The inability of the organism to survive at a slightly higher temperature, 37°C, indicates irreversible denaturation at this temperature.[12]

Other investigators[2,4,24,35,49] have also reported abnormally heat-sensitive enzymes in psychrophiles. For instance, moderate temperatures of 20°C to 30°C are sufficient to impair the ability of *Vibrio marinus* MP-1, an obligate psychrophile with a maximum growth temperature just under 20°C,

to respire endogenously and to utilize glucose. This implies that biochemical lesions in enzymatic pathways were induced by these temperatures.[33] Langridge and Morita[24] found that the malic dehydrogenase of this organism has a high thermolability and is readily inactivated at these low temperatures in cell-free extracts. The intact cell affords some protection, however, since the enzyme in growing cells is not so sensitive to inactivation by temperature variations. Similarly, Burton and Morita[4] found that malic dehydrogenase activity in a facultative psychrophilic marine vibrio, PS 207, was reduced to 40 to 45% of its original activity after just 15 minutes at 30°C, the maximum growth temperature of the organism. Heat denaturation was even more rapid at 35°C and 40°C. Again the whole cell afforded some protection against heat inactivation; possibly this greater thermal stability is due to the ability of the whole cell to renature damaged enzyme. Nevertheless, the enzyme's heat sensitivity at 30°C probably contributes to the inability of this psychrophile to grow above this temperature.[4]

Heat-sensitive enzyme synthesis. Upadhyay and Stokes[49] have compared the temperature sensitivity of the formic hydrogenlyase system from a facultatively anaerobic psychrophile, called strain 82, with that of the formic hydrogenlyase system from the mesophile, *E. coli*. This enzyme catalyzes the decomposition of the formic acid produced in sugar fermentations into hydrogen gas and carbon dioxide. The fact that the psychrophile produced a mixture of CO_2 and H_2 gases only at 20°C and below suggested that perhaps strain 82 contained a temperature-sensitive formic

hydrogenlyase or hydrogenlyase-forming system which is inactivated at temperatures above 20°C. Investigation revealed that both the enzyme and the enzyme-forming system were much more heat-sensitive in the psychrophile than in the mesophile. Formic hydrogenlyase was not found in cells of strain 82 which had been grown at temperatures greater than 20°C, but it was found in cells of *E. coli* over the mesophile's entire temperature range for growth, from 10°C to 46°C. The activity of the psychrophilic hydrogenlyase itself was maximal at 30°C but decreased at higher temperatures and was finally abolished at 45°C. In contrast, mesophilic hydrogenlyase activity was maximal at 45°C and was not completely abolished until a temperature of 70°C was reached.

Disorganization of the cell membrane. Thermal death may be due to the disorganization of the cell membrane and the resultant loss of control of permeability processes and leakage of materials from within the cell. Evidence has been presented by Morita and co-workers[31,32] which indicates that cell membrane damage can occur upon exposing an obligate psychrophile to moderate temperatures. Thirty minutes at 22.3°C induced leakage of material from within the cells of *Vibrio marinus* to the surrounding medium. This material included protein, DNA, RNA, and free amino acids, together with malic dehydrogenase and glucose-6-phosphate dehydrogenase. As dehydrogenases have been shown to be found in the protein portion of the bacterial membrane,[33] their presence in the medium implies breakdown of the cell membrane.

Just as the higher degree of unsaturation in the lipids of the

psychrophile may enable growth at temperatures lower than is possible for the mesophile by lowering the melting points of cellular lipids, so it may contribute to the lower maximum temperatures for growth of psychrophiles by enhancing disorganization of the lipids of the cell membrane at lower temperatures.

Hagen, Kushner, and Gibbons[11] studied an obligate psychrophilic marine bacterium in which temperature-induced death was followed by lysis. This organism does not grow at temperatures above 19°C and exposure of resting cells to 25°C causes a decrease in the turbidity of the suspension as well as leakage of 260 mμ-absorbing material to the seawater medium. There is a definite reduction in the lipid phosphorus shortly after this decrease in turbidity which results ultimately in the near-complete disappearance of this fraction, which is mainly phosphatidyl ethanolamine. The cause of death at this low temperature is not known, but lysis seems to be the result of activation of an enzyme, or enzymes, which causes the cell envelope to break up into fairly large particles. Since no soluble lytic enzyme was found, these investigators speculated that lysis might possibly be caused by a phosphatidase bound to the cytoplasmic membrane itself.

Accumulation of toxic products. It has been suggested that the accumulation of metabolic poisons within the cell is responsible for the low temperature maxima of psychrophiles.[33,43] Although this concept of autoinhibition of growth by toxic metabolic products is firmly established in the literature,[44] there is as yet little definitive evidence for its operation specifically in psychrophilic microorganisms.

SUMMARY

Most of the research concerning psychrophilic microorganisms has been aimed at discovering the biochemical bases for their ability to grow at very low temperatures and to do so at rates comparable to those of mesophiles at higher temperatures. Quite a variety of approaches has been taken to this problem. One of the first questions asked was how low temperatures affected biochemical reaction rates. The answer was not what one would expect from simple thermochemical reaction rate theory. With mesophiles and psychrophiles alike the Q_{10} values and energies of activation are greatly increased in the lower temperature ranges for their growth. Yet in the case of the psychrophile it appears that the temperature at which this increase takes place is lower. Several examples have been noted where the psychrophile's energy of activation for a given process also is lower than that of the mesophile for the same activity. Sultzer found this to be true for saturated fatty acid oxidation and Ingraham for glucose oxidation. Ng, Ingraham, and Marr suggested that derepression of enzyme synthesis in the mesophile at low temperatures is responsible for its higher energy of activation. Yet they did not suggest what importance this concept bears for the psychrophile.

Baxter and Gibbons attributed the ability of a psychrophilic species of *Candida* to respire glucose more rapidly at low temperatures than a

mesophilic species to the greater permeability of its cell membrane to glucose at these temperatures. They also suggested that the difference in permeabilities is due to the different lipids in the cell membrane, those of the psychrophile being more highly unsaturated than those of the mesophile. Kates and Hagen offered an explanation for this difference in the character of the lipids: the inability of the psychrophile to catalyze the transfer of a methylene group to a double bond to form cyclopropane acids from unsaturated acids.

Rose and Evison also presented evidence supporting the thesis that mesophiles are unable to grow at as low temperatures as psychrophiles because of their inability to transport sugars into the cell at such temperatures. But they attributed the difference in permeabilities to the restraining effect of low temperatures on the mesophile's protein molecules which, they suggested, were carriers involved in the transport process. Yet these investigators did not suggest how the psychrophile avoids similar restraints.

Research aimed at explaining why psychrophiles have relatively low *maximum* temperatures for growth has led to the discovery of enzymes and enzyme-forming systems which are exceptionally heat-sensitive and which are inactivated at low temperatures. Little has been done with the heat-sensitivity of the nucleic acids *in vivo*; consequently, their importance in limiting psychrophilic growth cannot yet be evaluated. However, their denaturation temperatures are such that it does not seem likely that they are involved in determining the maximum growth temperatures for

psychrophiles. The same high degree of unsaturation of the lipids which was assumed to permit the psychrophile to function at very low temperatures has also been implicated in causing cell death at relatively low temperatures by contributing to the disorganization of the cell membrane. There is also evidence that the psychrophile loses control over permeability at low temperatures and that the subsequent leakage of cell materials leads to death.

CONCLUSION

Truly psychrophilic microorganisms do exist and are distinguished by their ability to grow at very low temperatures and to do so at rates comparable to those of mesophiles at higher temperatures. Therefore, the kinetic rate constants for the bio-oxidation of organics by psychrophiles under arctic conditions may not be radically different from those found for mesophiles under more temperate conditions. Consequently, it is suggested that it is not valid to extrapolate from data obtained with mesophiles subjected to extreme cold to a situation where psychrophilic microorganisms are involved.

Such thoughts lead one to suspect that biological waste treatment in the North might not be so difficult as one would suppose. It is hoped that this review will further the research effort along these lines, because the increasing populations in all northern areas necessitate that definite criteria be established concerning waste treatment practices and that basic information be obtained on the ability of our waters to assimilate our wasteproducts.

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