

A Role for Water in Growth, Metabolism, and Intracellular Organization¹

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Evidence for the existence of vicinal (interfacial) water is reviewed briefly and its role in cellular functioning is outlined. In the present study, data are reported for the growth of a thermophilic alga, Cyanidium caldarium, as a function of temperature. Several growth optima and minima were observed; the growth minima generally occurring at the temperatures where the vicinal water undergoes structural changes. When cultured on a minimal medium in the presence of NH_4^+ as the nitrogen source the organism exhibited a growth peak above 45°C . However, when the NH_4^+ was replaced by NO_3^- , no growth occurred above 45°C . This result is discussed in terms of our theory of multiple metabolic pathways, determined in part by the properties of vicinal water.

For some microorganisms cultured over wide temperature ranges, the occurrence of multiple growth optima has been reported sporadically in the literature. A typical example of such multiple growth optima is shown in Figure 1 from a study by Mitchell and Houlahan (1). These authors measured the growth of a mutant of *Neurospora crassa* which required riboflavin for optimum growth. It is seen from Figure 1 that at reduced levels of riboflavin, a distinct minimum in the growth curve occurs near 30°C . In previous studies (2, 3, 4) the senior author has

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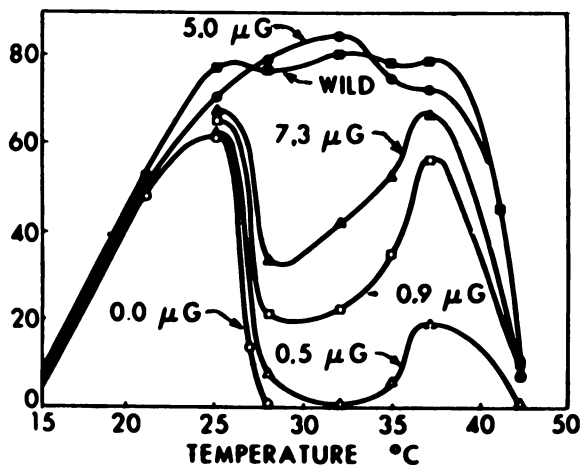


Figure 1. Multiple growth optima for a mold (a mutant of *Neurospora crassa*) as a function of temperature with different amounts of riboflavin present (1)

proposed that minima in growth curves reflect structural transitions of the intracellular water. In this chapter, we discuss some aspects of multiple temperature optima and particularly the suggestion that maxima and minima in the growth processes may reflect different metabolic pathways, the selection of which depends on the structure of the vicinal, intracellular water.

Before proceeding, we wish to summarize some of the observations which have been reported in the literature on vicinal water and to illustrate the likely role of such water in cellular functioning through a number of examples, mostly taken from the literature.

Summary of Vicinal Water Properties

Water and aqueous solutions adjacent to most (solid) interfaces possess notably different properties from those of the respective bulk systems. Such interfacial water is referred to as vicinal water. Because the properties of vicinal water differ from the properties of bulk aqueous systems, it must be concluded that the structure of vicinal water differs from the bulk structure (5, 6, 7, 8, 9). The evidence presently available also suggests that the modification of the structure extends over considerable distances, apparently as much as 100 to 1000 Å or roughly 30 to 300 molecular diameters. The evidence for extensive structure modification derives in part from the following types of measurements: (a) disjoining pressure (10, 11); (b) viscosity (11, 12, 14); (c) ion selectivity (14, 15); (d) ultrasonic absorption and velocities (16, 17); (e) dielectric data

(18, 19, 20, 21, 22); (f) conductance data (23, 24); and (g) ultraslow mechanical relaxation (shear) (23, 24, 25). Additional evidence is reviewed in Refs. 6 and 7, while Ref. 8 should be consulted for a collection of recent contributions towards the elucidation of the role of vicinal water in cellular systems.

In this chapter we discuss some specific aspects of vicinal water as it affects intracellular organization and, in particular, the effects on metabolic pathways and growth rates.

Thermal Anomalies: Paradoxical Effect. The properties of vicinal water exhibit thermal anomalies, manifested as more or less abrupt changes in the temperature coefficient of those properties over at least four different temperature intervals, specifically near 14° to 16°C, 29° to 32°C, 44° to 46°C, and 59° to 62°C (5, 6, 7). Another unique aspect of vicinal water is the so-called "paradoxical effect" (7): it appears, to a first approximation, that the temperatures of the thermal transitions (T_k) are independent of the chemical nature of the solid surfaces and relatively independent of the concentration and nature of the solutes in the aqueous phase.

Structural Aspects. The structure of vicinal water is not known. In fact, it is not even clear if vicinal water is "more structured" or "less structured" than bulk water. Possible types of vicinal water structures are clathrates and high-pressure ice polymorphs (6, 7). However, it is possible also that no definite new types of structures occur in vicinal water although the existence of multiple thermal transitions strongly suggests that definite, relatively large-scale, cooperative effects must play a role—something which is difficult to envision if identifiable structured entities do not exist.

Molecular-Weight Effects. Vicinal water appears to occur at most (or all) aqueous phase/solid surfaces. Hence, the effects of vicinal water must be expected to be manifested in cellular systems because of such interfaces as the cytoplasm/cell membrane or the interface at various organelles. However, it is important to note that vicinal water also occurs in aqueous solutions of macromolecules (7, 8). Thus, there is further reason to expect that cellular systems should reveal the effects of vicinal water.

Effects of Vicinal Water on Growth Processes

Illustrative Examples. The evidence for the occurrence of—and a role for—vicinal water in cells is derived from a variety of observations on living systems. One of the most useful approaches for demonstrating the existence of vicinal water has been through studies of thermal effects: the frequent occurrence in cellular systems of thermal anomalies (at the

transition temperatures of vicinal water) strongly implicates vicinal water in some rate-determining processes in cells. A few examples of thermal effects in living systems are reviewed in this section.

Figure 2 shows a typical example of the abruptness with which a large number of cellular systems respond to temperatures near the vicinal water transition temperatures. The mean germination period (measured in hours) for freshly harvested as well as "after-ripened" seeds of barley (var. Golden Promise) as reported by Roberts and Smith (26) is plotted in this graph. A remarkable and sudden retardation of the germination

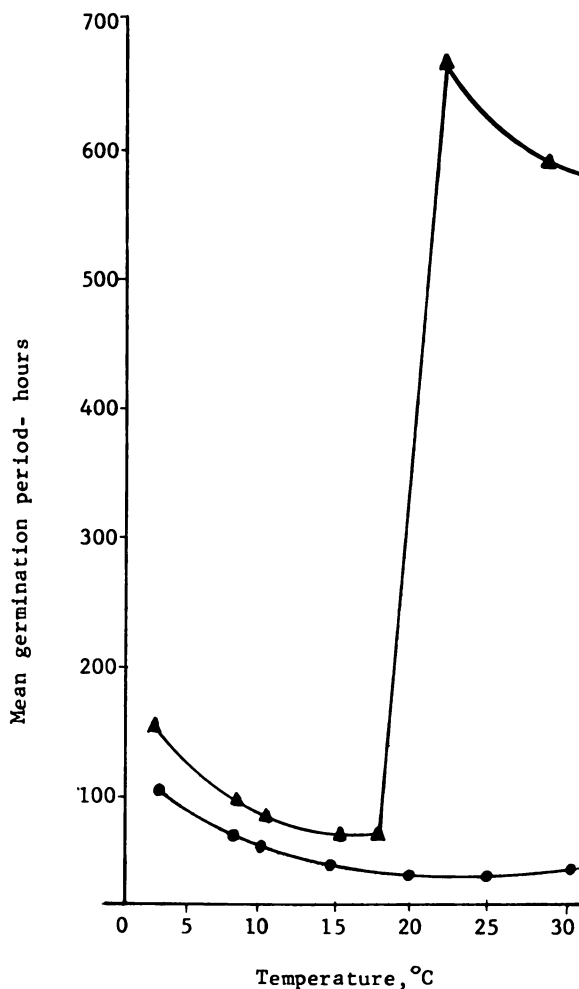


Figure 2. Mean germination period of freshly harvested (▲) and after-ripened (●) barley seeds as a function of temperature (21)

occurs above approximately 16°C. (See also Ref. 6 for a discussion of the possible role of vicinal water in vernalization.)

Figure 3 shows the effects of temperature on the survival of the green photosynthesizing macroalga *Valonia macrophyssa* (27). The domain of survival (for 3-days exposure) is limited sharply by the temperatures of two of the vicinal water transition temperatures, namely 15° and 30°C. Thorhaug (27) has provided many additional examples of such thermal boundaries (see also Ref. 28).

The example shown in Figure 3 clearly suggests that a temperature "domain" exists, with sharply defined boundaries, in which the organism can function. For other organisms, a different domain might be between 30° and 45°C. At each of these boundaries rapid changes with temperature are expected in a variety of cell functions; as examples, membrane permeabilities and membrane resistances may change (9) and a large number of enzymatic reactions may be affected (6, 7, 29), etc. This in turn has led to various theories to explain a number of physiological phenomena. As an example, Drost-Hansen (6, 30, 31) proposed earlier that body temperatures of mammals and some birds have been chosen through evolutionary processes to be as far away from either of these T_k 's (30° and 45°C) as possible—i.e., body temperatures are near 37°C (98.6°F) which is indeed close to the average body temperature of about 150 mammals and those birds which do not fly (ostrich, penguin, kiwi, etc.). Other implications are discussed in Refs. 6, 8, and 31.

Finally, Figure 4 shows the effects of temperature on the rate of germination of turnip seeds. The ordinate in this figure is the logarithm of the reciprocal of the time (in hours) for 50% germination, plotted as a function of the reciprocal absolute temperature. Anomalous changes in slope occur near 15° and 30°C. A detailed discussion of these data can be found in Refs. 32 and 33.

Many examples can be added to demonstrate the occurrence of remarkably abrupt changes in physiological parameters (including growth) at the vicinal water transition temperatures (6, 7, 8, 28, 29, 33, 34). The available data are not restricted to plants and algae which have been used here only to demonstrate the nature of the phenomenon.

Mechanism of Control by Vicinal Water in Cellular Functioning. Vicinal water appears to exist adjacent to (most) solid/aqueous interfaces (4). These solid/water interfaces include membrane systems (9, 34) and the interface of dissolved biomacromolecules with the aqueous phase (29). The effects of vicinal water are also frequently apparent in enzyme kinetic data in bulk solution (i.e., in vitro; away from any biological surfaces (29, 32); and in enzyme data in living cellular systems).

In the case of isolated membrane systems, it is not difficult to imagine a direct functional role for vicinal water. Changes in permeability and/or

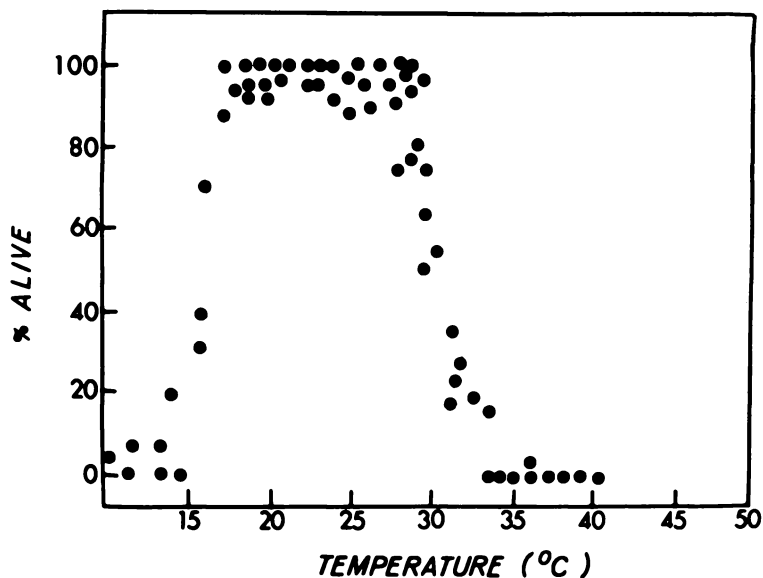


Figure 3. Survival of *Valonia macrophysa* (a green macroalga) as a function of temperature after 3 days. Each point represents between 25 to 100 cells (22).

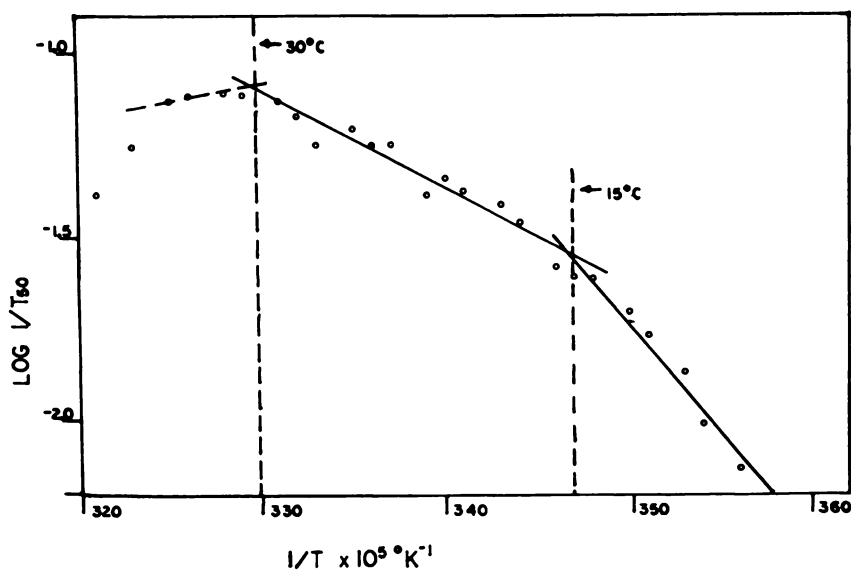


Figure 4. Effects of temperature on germination of turnip seeds (Arrhenius plot). Log 1/time to 50% germination vs. 1/temperature. See text for details.

ionic activities have been discussed (8,9). However, for the occurrence of thermal anomalies in many processes of intact cells, it is far more difficult to identify a site of action. Nonetheless, one important suggestion has been made recently by Clegg (35) who consider the possibility that the majority of the cellular macromolecules function primarily in the vicinally ordered water of various intracellular interfaces rather than in a bulk aqueous phase. This chapter should be consulted for some stimulating thoughts on the subject.

Multiple Growth Optima and Multiple Metabolic Pathways

Multiple Growth Optima. Earlier in this chapter, a few examples were discussed which illustrate the abrupt changes sometimes observed in cellular functioning at the vicinal water transition temperatures (T_k). In many cases, the T_k 's correspond to absolute upper thermal limits for organisms (*see* especially Refs. 6, 28, and 29). However, in some cases, particularly for single-cell organisms, multiple growth optima have been observed. In these instances, the region around one (or more) T_k is associated with partial or complete suppression of growth, while at both higher and lower temperatures, growth does occur. A few illustrative examples are discussed below.

Figure 5 shows a typical example of multiple growth optima. The ordinate is the amount of growth (after 16 hr) of *Streptococcus faecalis*, as a function of temperature (36, 37, 38). A distinct growth minimum occurs near 29°C.

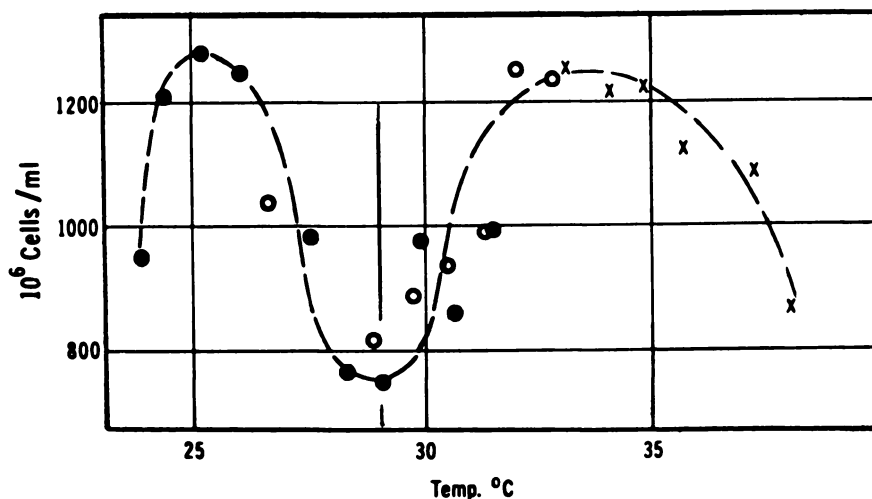


Figure 5. Number of cells of *Streptococcus faecalis* as a function of temperature (40)

Another example is shown in Figure 6 in which minima in growth rate are reported for two varieties of beets (39). Again, distinct minima are observed in the growth of the two varieties, namely between 11° and 14°C and ca. 14° to 16°C, respectively—in other words, near T_k 's ca. 14° to 16°C. Note also the cessation of growth in the range between 28° to 31°C.

Other examples of multiple growth optima have been discussed in some of the literature referred to earlier. In the next section we discuss a tentative hypothesis to explain the occurrence of bimodal (and multimodal) growth optima.

Multiple Metabolic Pathways. In discussing the occurrence of multiple growth optima, Oppenheimer and Drost-Hansen (2) proposed that different metabolic pathways were utilized in different temperature regions (i.e., in the regions between T_k 's) owing to some structure-dependent processes related to water-structure transitions.

An attempt to elucidate such processes was made by Schmidt and Drost-Hansen (3,4) who measured pH changes accompanying the growth of *E. coli* over a wide temperature range. Depending on the

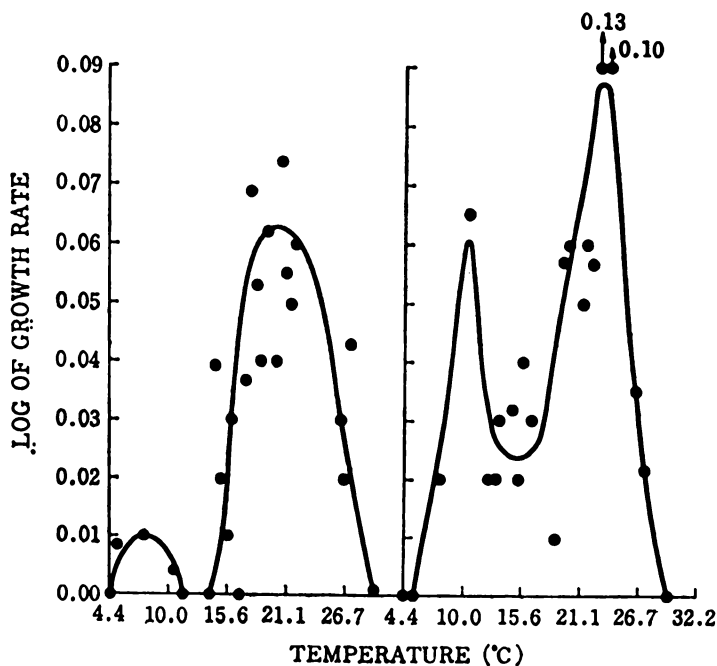


Figure 6. Plot of logarithm of growth rate for two varieties of beets as a function of temperature (41)

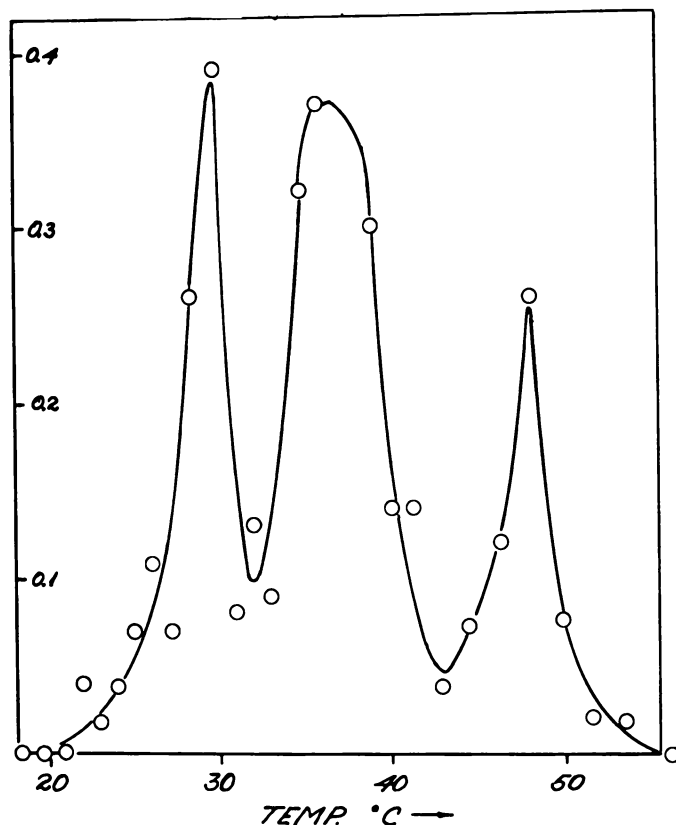


Figure 7. Growth of *Cyanidium caldarium* (grown on NH_4^+ -containing merium) as a function of temperature. See text for details.

temperature, either increases or decreases in pH were observed. However, while the results obtained were entirely consistent with the proposal by Oppenheimer and Drost-Hansen (2), the experiments did not prove a cause-effect relationship between (vicinal) water structure changes and the selection of one specific metabolic pathway over another.

Recently, Etzler and Drost-Hansen (32) have re-examined the question of possible mechanisms for multiple growth optima. In this study, the rate of growth of a green thermophilic photosynthesizing alga (*Cyanidium caldarium*) was measured (at closely spaced temperatures—see Ref. 40). Figure 7 shows the amount of growth observed after 21 days as a function of temperature. One observes no less than three clearly separated growth optima with distinct minima near 32° and 43°C —in good agreement with expectations from the theory, according to which temperature regions around T_k lead to impaired cell functioning.

An Example of Metabolic Pathway Selection

Multiple growth optima are observed most frequently for growth on minimal media (2, 3, 6; see also Ref. 29). In the example discussed above, the growth of *Cyanidium caldarium* did indeed take place in a minimal medium: nitrogen was provided in the form of $(\text{NH}_4)_2\text{SO}_4$; in addition, the medium contained KH_2PO_4 , MgSO_4 , CaCl_2 , FeSO_4 , H_3BO_3 , and trace amounts of Zn, Mn, Ni, Co, and H_2SO_4 (pH ~ 2). However, no other solutes, organic or inorganic, were added to the medium. Atmospheric CO_2 served as the only carbon source!

In a more recent series of experiments, the alga (*Cyanidium caldarium*) was grown in the same medium, only modified to contain NO_3^- instead of NH_4^+ as the nitrogen source. The result is shown in Figure 8.

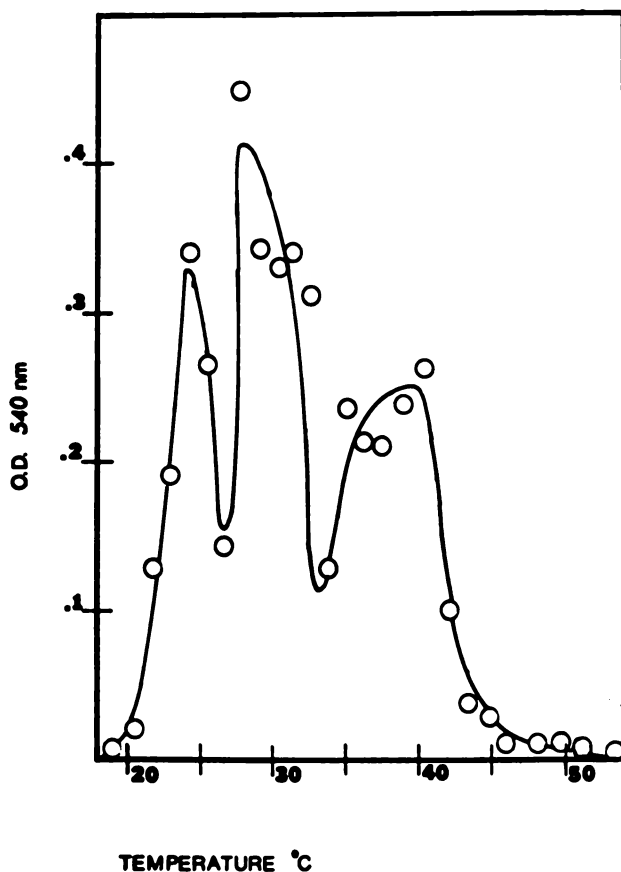


Figure 8. Growth of *Cyanidium caldarium* (grown on NO_3^- -containing medium) as a function of temperature. See text for details.

It is apparent from these data that growth did not occur above 45°C—as opposed to the results obtained with the NH_4^+ -containing medium in which a growth peak occurred at a higher temperature, namely ca. 50°C. It appears that a change has taken place in the choice of metabolic pathways. NO_3^- apparently cannot be utilized above 45°C—i.e., above T_k (the temperature of the vicinal water transition). As in the experiments by Schmidt and Drost-Hansen, the result is consistent with the hypothesis of a controlling role for vicinal water in cellular functioning but again it does not prove this point. On the other hand, we are unable to propose any alternative explanation, particularly considering the diversity of systems in which different metabolic requirements appear modified upon crossing a vicinal water transition temperature. We stress that it may be possible to construct other mechanisms to explain multiple growth optima and that it is difficult—or impossible—at this time to identify specific molecular processes, involving vicinal water, which readily can explain the phenomenon. On the other hand, the abruptness of change of growth rates which often is observed and the conspicuous role played by temperatures near T_k do lend credence to the notion of a likely role of vicinal water in multiple growth optima. On this basis then it seems reasonable to propose that the mechanism may be explained in terms of a choice between different, allowable metabolic pathways.

Summary

Vicinal water occurs near most interfaces between a solid and an aqueous phase. The vicinal water appears to exert profound effects on some regulatory processes in cellular systems. The examples discussed included various rate processes such as growth, body temperatures, upper (lethal) thermal limits, and rates of germination. In addition, vicinal hydration effects appear to influence other cell processes, including the choice of metabolic pathways. This is suggested strongly by the results reported in this chapter, showing a thermophilic alga (*Cyanidium caldarium*) able to grow above 45°C in the presence of NH_4^+ but not with NO_3^- as the only nitrogen source.

Acknowledgments

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