

Water is the only chemical that exists naturally in our ecosphere in all three states of matter. It is therefore of interest to enquire into the interconversion mechanisms between gas, liquid and solid in the ecosphere. The hydrological cycle of evaporation followed by precipitation recycles the Earth's total accessible liquid water resources 37 times annually, which demonstrates the vastness of our 'natural water-purification plant' and water as a limited but non-depleting resource (Franks 2000).

The issue of phase transformations becomes of even greater significance, where such transitions impact directly on life processes, because it is now well recognized that low temperature and, particularly, freezing are the fiercest environmental enemies of many species. As regards the effects on living organisms, a clear distinction is to be drawn between 'low temperature' and 'freezing' (Franks 1985). The former expression describes purely biological changes produced by changes in the properties of *liquid* water with temperature, e.g. the density maximum at 4 °C. These effects are frequently described as 'chill.' Freezing, on the other hand, involves the removal of water from the organism, in the form of ice, as a separate phase. It therefore leads to dramatic changes in the concentrations of all soluble substances in the cytoplasm. Freezing is thus classified as an osmotic stress condition, along with drought and salinity. A vast literature catalogues different types of physiological and metabolic injuries that arise from osmotic stresses, which are in reality just water stresses. The fact that most living organisms exhibit optimum growth in aqueous environments lying between relative humidity values of 99.9% and 99.999% (corresponding to an osmolality of 0.15 Osm l⁻¹ or a water potential of -10⁻⁴ Pa) illustrates the extreme sensitivity of the coupling of life processes to the aqueous environment.

Any conditions outside this very limited optimum range, however produced, are perceived as water stresses, which the organism can either resist up to a point or to which it has developed the necessary genetic and biochemical adaptation machinery. Freeze resistance and freeze tolerance must therefore be treated as quite distinct ecological processes. Freeze-resistance mechanisms render the organism capable of existing unfrozen at sub-zero temperatures, where the bodily fluids can exist for long periods in an undercooled state, i.e. under highly non-equilibrium conditions. Freeze-tolerant species can, under certain conditions, survive seasonal or even quite short-term changes in their osmotic environment. They always require an environmental 'early warning' that such changes are about to occur; this warning allows them to put in motion a variety of adaptation processes (Franks 1985).

At a 1989 Royal Society Discussion Meeting entitled 'Life at low temperatures', a group of physical, protein and lipid chemists, enzymologists, plant and marine physiologists and microbiologists surveyed the many aspects of terrestrial low temperatures, their impact on living species and the variety of ways in which they respond to the stresses. Nucleation of ice and other chemical components featured only peripherally in the proceedings, but their importance was recognized. They form the centrepiece of this article.

2. Nucleation theory as applied to ice

Classical nucleation theory applies equilibrium thermodynamics to a model of step-wise atomic or molecular aggregation until an embryo of critical size has been reached that is capable of spontaneous growth to form a daughter phase within the pre-existing mother phase (Debenedetti 1996). Nucleation is viewed as a stochastic pro-

Table 1. Undercooling capacity of molecular liquids

liquid	T_h/T_m
water	0.85
methyl bromide	0.86
phosphorus	0.79
ammonia	0.75
sulphur dioxide	0.83

cess, where the probability of an embryo of critical size being formed depends on the total number of molecules and the lifetime of the embryo. As is the case for other physical processes, nucleation calculations are often based on interactions between spherical particles. This would seem to be inappropriate for water, which in the condensed phases and at normal pressures exists as a four-coordinated structure. By virtue of its sp^3 hybridization, its condensed phases are able to form more-or-less distorted three-dimensional networks, the molecules being held together (or apart) by weak bonds of a very narrow range of lengths and angles. Unlike covalent bonds, these weak hydrogen bonds are also subject to rapid exchange, even in the solid phase.

The conventional model of stepwise molecular aggregation might be valid for the nucleation of water or ice in supersaturated water vapour, as it occurs in the upper atmosphere. However, the ability of the model to account for the nucleation of ice in undercooled liquid water or in supersaturated aqueous solutions must be open to doubt. It has for many years been accepted that liquid water possesses several ice-like features at low temperatures. In particular, the fusion of ice is accompanied by only a very minor change in the coordination number, from 4.0 to 4.4. Recent neutron-diffraction studies on liquid water have reinforced prior evidence, based mainly on X-ray diffraction and thermodynamic comparisons of the liquid and ice. The neutron data are particularly significant, because they shed light on the configurational part of the molecular-pair distribution function which had been previously inaccessible to experimental study (Finney & Soper 1994). If, therefore, the liquid already possesses important features of the solid phase to be nucleated, then the degree to which liquid water can be undercooled is surprising, even though it is of the same order of magnitude as is found for many other molecular liquids. The undercooling ratios of some typical liquids are shown in table 1 (Franks 1985). A further weakness of the classical theory lies in the assumption that physical properties, e.g. the structure and surface free energy, of the growing embryo can be equated to those of the solid crystalline phase, hexagonal ice (ice Ih).

Experimental tests of theoretical predictions have been confined mainly to nucleation from the vapour phase. In order to study homogenous nucleation of solids from supersaturated liquid samples, any possible dust impurities must be rigorously excluded, since they might act as heterogeneous catalysts. This is best achieved by dividing the sample into many micrometre-sized subsamples such that only a few of these compartments could suffer from contamination by dust particles. One experimental approach is the employment of emulsified samples, where small aqueous droplets are emulsified in an inert oil matrix. The nucleation process is best measured by the heat of crystallization liberated by either isothermal or scanning calorimetry.

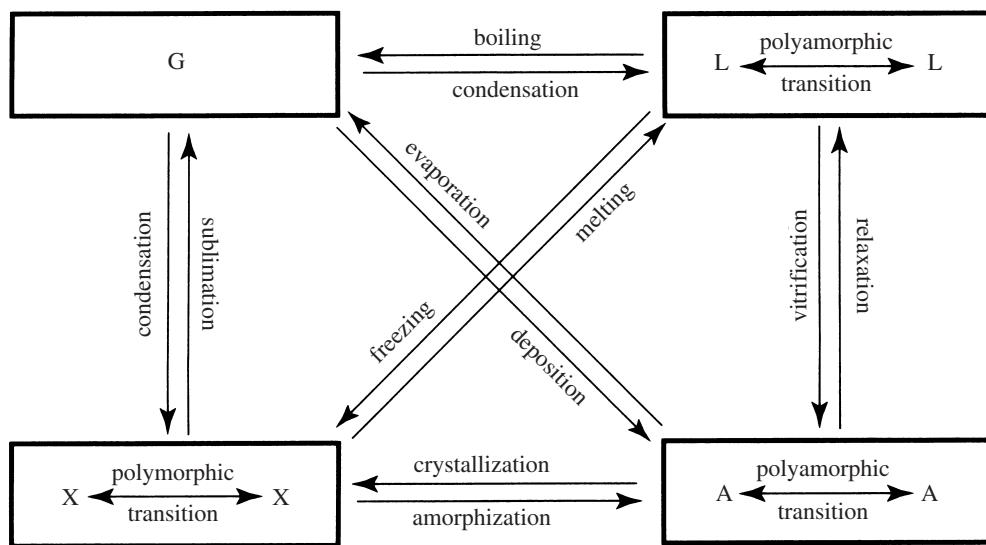


Figure 1. Phase transitions between equilibrium gas (G), liquid (L) and crystalline (X) water phases. Also included are metastable amorphous states (A), where the transitions depend on experimental conditions. (Reproduced with permission from Mishima & Stanley (1998).)

Care must, however, be taken to demonstrate that only a single nucleation event occurs in each droplet. In other words, each droplet must yield a single ice crystal, so that the heat output can be unambiguously related to the number of nucleated droplets. An additional advantage of the technique is that more than one million droplets can be monitored in a single experimental run, thus providing very good statistics and reproducibility (Michelmore & Franks 1982).

3. Nucleation of ice in the liquid phase: experimental results

There have been more and more studies of ‘liquid’ water at sub-zero temperatures in recent years. While the many polymorphic crystalline ice forms have been studied extensively, the fast-quench cooling of liquid water to very low temperatures has revealed novel classes of water modification, based on amorphous solid (vitreous) water. The multiplicity and complexities of the various possible transitions between condensed phases, even just at atmospheric pressure, are illustrated in figure 1 (Mishima & Stanley 1998).

The mysteries have been further compounded by the discovery that, depending on the method of preparation, two distinct disordered, glassy water forms differing in density can be produced, giving rise to the interesting phenomenon of polyamorphism, i.e. the existence or even coexistence of two distinct disordered phases. More recently it has been shown that, by performing the cooling under moderately raised pressures (*ca.* 0.5 kPa), water can be vitrified to the high-density form even without the need for ultrarapid cooling (Mishima & Suzuki 2001). On warming, a sharp transition, accompanied by a volume expansion, is observed at *ca.* 130 K. This corresponds to the characteristic transition of the high-density state to the low-density

Table 2. *Distribution of ice nuclei within dispersed water droplets*
(Computed from data by Dufour & Defay (1963).)

temperature (K)	drop radius (nm)	droplets per gram	nuclei containing a nucleus	proportion of droplets
263	10	2.4×10^{17}	2×10^{-433}	8.4×10^{-449}
253	10	2.4×10^{17}	8.2×10^{-75}	3.4×10^{-92}
	5	1.9×10^{18}	2.5×10^{-93}	1.3×10^{-111}
243	10	2.4×10^{17}	2.5×10^{-18}	1.1×10^{-35}
	5	1.9×10^{18}	1.4×10^{-23}	7.2×10^{-42}
233	10	2.4×10^{17}	6.3	2.6×10^{-17}
	5	1.9×10^{18}	2.9×10^{-2}	1.5×10^{-20}

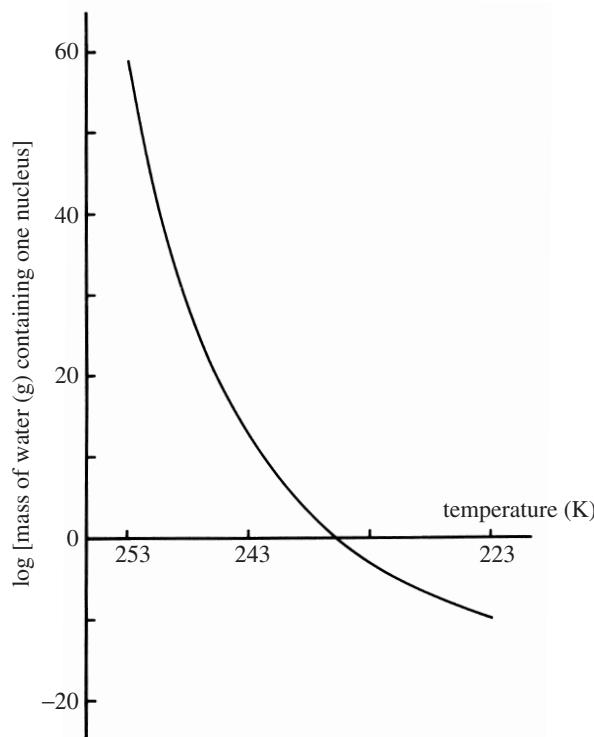


Figure 2. Mass of water (in grams) likely to contain one critical-sized nucleus as a function of temperature, assuming homogeneous nucleation. (Adapted with permission from Franks (1985).)

state of amorphous ice. Further warming results in the crystallization of cubic ice (ice Ic) at 160–170 K.

Although a discussion of the physical properties of these remarkable substances is beyond the scope of this article, presumably nucleation phenomena must play a central role in the various possible disorder-to-order transformations shown in figure 1, and probably even in polyamorphic transitions. The properties of water

clusters and the dimensions of critical nuclei have been computed and tabulated as functions of droplet radius and temperature (Dufour & Defay 1963). For studies based on the droplet-emulsion technique, it is possible to calculate the mass of water which is likely to contain one critical nucleus at a given temperature; the results are shown in figure 2. It is seen that, around 240 K, a temperature is reached where every gram of water is likely to contain one nucleus capable of triggering the growth of an ice crystal. If the mass of water is divided into droplets with a mean radius of, say, 5–10 nm, it becomes possible to estimate the proportion of drops that are likely to contain a critical nucleus and will therefore freeze at that temperature. This is illustrated in table 2 for several temperatures (Franks 1985). Nucleation is seen to be extremely sensitive to changes in temperature and the very large numbers involved in the calculations change rapidly over small ranges of temperature.

It is also important to consider the kinetics of nucleation. Transition-state theory is usually employed for this purpose, the process being viewed as a first-order addition of single molecules to the growing embryo, as mentioned above. The free energy of self-diffusion of undercooled water is used in the calculations as a quasi-activation energy (Fletcher 1970). The nucleation rate J can be adequately expressed by

$$J = A \exp(B\tau),$$

where $\tau = [T^3(\Delta T)^2]^{-1}$ and ΔT is the degree of undercooling.

For comparison of nucleation rates in different systems, an even more useful representation of J can be derived, with the aid of the reduced temperatures

$$\theta = \frac{T}{T_m}, \quad \Delta\theta = \frac{T_m - T}{T_m},$$

where T_m is the equilibrium melting (freezing) temperature. The inverse fifth-order temperature dependence of the rate indicates that J increases sharply over a narrow temperature range (Michelmore & Franks 1982). The experimentally accessible range is limited; figure 3 combines the experimental results with those calculated from the ‘best’ data (at the time) for undercooled water (Franks *et al.* 1984). It is seen that, for the highest experimentally achievable values of ΔT , the nucleation rate J ($\text{m}^3 \text{s}^{-1}$) increases by a factor of $20 \text{ }^\circ\text{C}^{-1}$ and it passes through a maximum at *ca.* 40 °C. Homogeneous nucleation is therefore a well-defined event and does not depend greatly on the cooling rate. Turning once more to ecological implications, the homogeneous nucleation temperature T_h (*ca.* –40 °C) must be regarded as the absolute limit to the phenomenon of biological freeze resistance.

The ability of water to undercool can be considerably enhanced by the application of pressure, as illustrated in figure 4. A comparison of the solid–liquid equilibrium phase diagram with the nucleation profile demonstrates two surprising relationships between the equilibrium freezing temperature T_m and T_h . For every degree of depression in T_m , T_h is depressed by two degrees, although there is no obvious formal relationship between freezing and nucleation. The other remarkable feature in figure 4 is the effect of pressures above 200 MPa, which corresponds to the ice I–ice III transition. Here too, $T_h(P)$ mirrors the phase coexistence curve, and the ternary eutectic point for liquid–ice I–ice III is exactly reflected in the nucleation curve.

Turning now to the homogeneous nucleation of ice in aqueous solutions, an identical parallelism exists between the freezing point and nucleation temperature, as

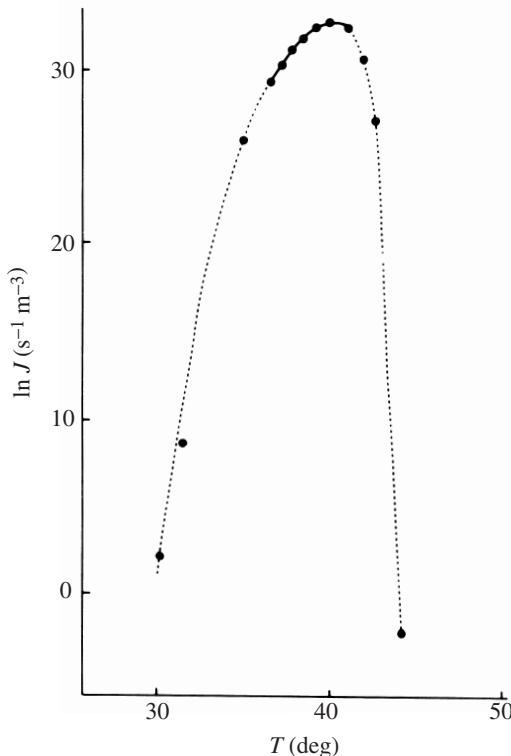


Figure 3. Nucleation rate of ice in water as a function of the degree of undercooling. The solid line represents the experimentally accessible region; calculated values (dots) (Franks *et al.* 1984) are joined by the broken curve. (Reproduced with permission from Franks *et al.* (1984).)

illustrated by the corresponding Clausius–Clapeyron plots in figure 5. Here again the nucleation-point depression is double the freezing-point depression. It is also noteworthy that the $T_h(x_2)$ profile, where x_2 is the molar-fraction solute concentration, is insensitive to the chemical nature of the solute (Rasmussen & MacKenzie 1972). It is seen to depend solely on x_2 , which is itself related to the water activity a_w by $a_w = p/p^0$, the saturation vapour-pressure ratio of water in the solution to that of pure water. The above observations lead to the conclusion that nucleation, although itself a kinetic-rate process, can be modelled by equilibrium thermodynamics. Such a thermodynamic theory for homogeneous ice nucleation in terms of a_w has recently been proposed (Koop *et al.* 2000) and has been shown to be a reasonable explanation for the nucleation processes observed in clouds.

In most practical situations, e.g. in living cells and in the upper atmosphere, ice nucleation is catalysed by extraneous matter, so that it takes place at temperatures higher than -40°C . The process is then referred to as heterogeneous nucleation. It must, however, be emphasized that the actual structure of the ice nucleus is believed to be identical to that produced during homogeneous nucleation. The discovery of biogenic nucleation catalysts and inhibitors led to intensive studies of undercooling and nucleation phenomena of ecological (and technological) significance, some of which will be discussed in the following pages.

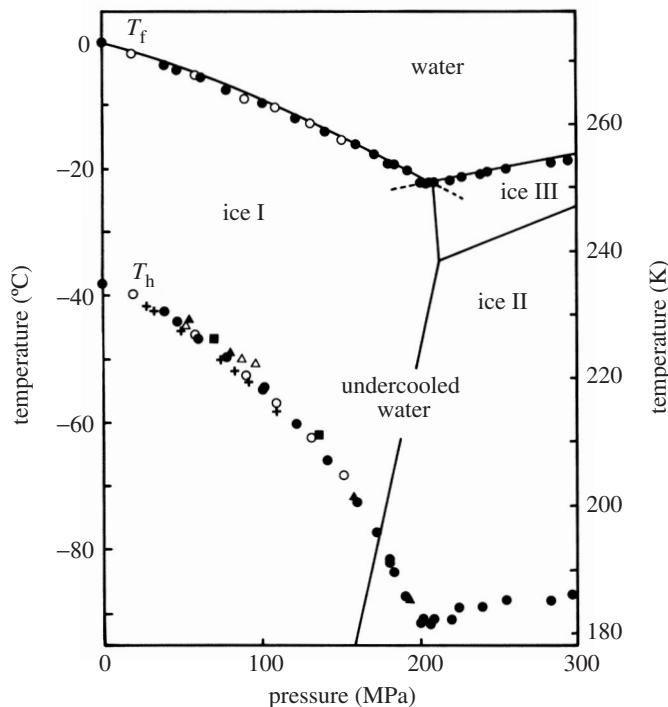


Figure 4. The effect of pressure on the melting point and the homogeneous nucleation temperature of ice in undercooled water. (Adapted from Kanno *et al.* (1975).)

4. Extraneous ice-nucleation catalysts

Although the phenomenon of heterogeneous, or facilitated nucleation, as applied to ice, has long been known (Hobbs 1974), uncertainty still exists about the structural requirements that would render a solid surface effective as an ice-nucleation catalyst. Originally it was believed that the wetting characteristics (contact angle) of the solid determined its catalytic efficiency. It was also believed that a structural match must exist between the ice crystal and the catalyst. This belief led to the many studies of ice nucleation catalysed by silver iodide, even to the extent of using AgI in a pilot project designed to enhance the snowfall over the Rocky Mountains in efforts to raise the water level of the Colorado River (Weisbecker 1967). The project, although found to be technologically feasible, was abandoned for politico-legal reasons. More recently, the catalytic efficiency of AgI has been assigned, not to a crystallographic match with ice, but to crystal dislocations and/or trace impurities, i.e. a mismatch. Indeed, it has been reported that AgI, doped with a small amount of AgBr, is a more effective catalyst (Vonnegut & Chessin 1971). The present view of what forms a ‘good’ nucleation catalyst is based on three requirements: it should have a small lattice mismatch with ice; it should have a low surface charge; and it should possess a degree of hydrophobicity, i.e. it is not wetted by water.

Ice formation in the upper atmosphere can be successfully accounted for in terms of homogeneous ice nucleation in aqueous sulphuric acid droplets. On the other hand, heterogeneous nucleation must also play an important role in the condensation of supersaturated water vapour in the upper atmosphere, especially in regions of indus-

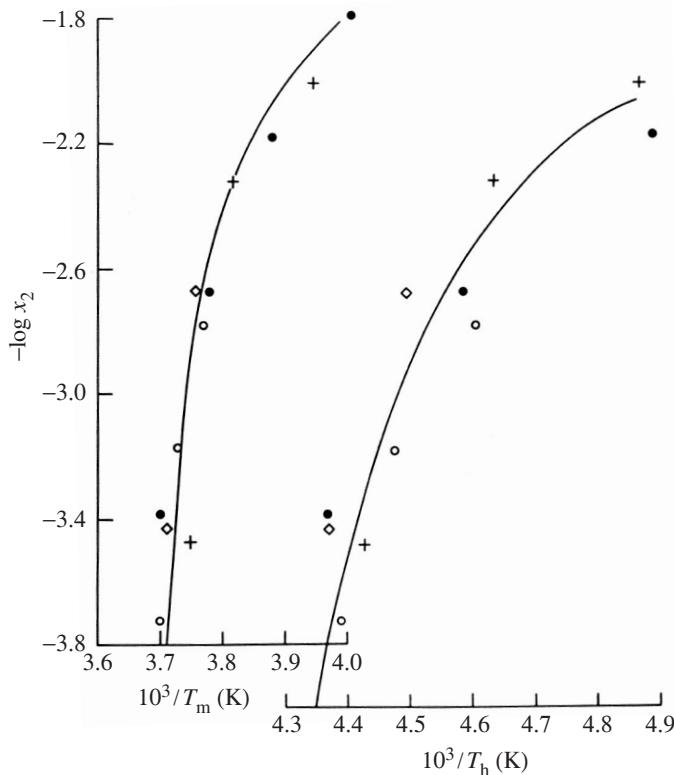


Figure 5. Clapeyron–Clausius plots of melting points and homogeneous nucleation temperatures of aqueous solutions as functions of solute concentration. Closed circles, ethane glycol; open circles, glucose; open diamonds, urea; crosses, NaCl. (Adapted with permission from Franks (1982).)

trial activity and the resulting air pollution. Atmospheric aerosol particles are solid or liquid particles suspended in air. Processes that control the formation, transformation and removal of atmospheric aerosols are of great interest in atmospheric science. The particles, often less than $1\text{ }\mu\text{m}$ in diameter, affect Earth's radiation budget through the scattering of sunlight and through their interaction with clouds. Burning of fossil fuels and changes in the use of land affect the properties of the aerosol and may therefore influence the climate, either directly through an increase in the number of aerosols or indirectly through the way in which the aerosols change the mechanisms of cloud formation. An example is provided by the formation and persistence of cirrus clouds, which exist in the upper troposphere at altitudes of 7–17 km. They are composed of non-spherical ice particles with particle sizes and number densities varying considerably with meteorological conditions and origin of the air in which they form. Recent evidence supports a contribution of homogeneous freezing in the formation of cirrus at very low temperatures. It has also been suggested that haze particles (concentrated solution droplets of subcritical dimensions for growth at the prevailing humidity) may freeze directly from the vapour state. Field evidence about ice formation in cirrus and in wave clouds at temperatures close to the homogeneous nucleation temperature of water, obtained by direct sam-

pling from aircraft (Sassen & Dodd 1988; Heymsfield & Miloshevich 1995; Jensen *et al.* 1994), have led to improved nucleation models and improved prediction methods. Because cirrus clouds cover up to 30% of the Earth, they play an important role in atmospheric chemistry and climatic conditions. They also exert a net heating effect on the Earth, because they only marginally affect the amount of incoming visible light from the Sun but efficiently absorb the outgoing infrared radiation from the Earth's surface, part of which they re-emit back to the ground. Even small changes in cirrus-cloud coverage may significantly alter the Earth's climate. However, since the exact formation mechanisms for cirrus clouds are still unknown, it is difficult to predict how today's human activities might change cloud abundance in the future.

An improved understanding of life cycles and transport patterns of aerosol particles and of cloud-formation mechanisms requires adequate models of nucleation, but these are not yet available. However, even if such an understanding were to be achieved, an effective control of such nucleation processes in the upper atmosphere could hardly be achieved, except when based on comprehensive global agreements. At present, the prognosis for success must be rated as bleak. By comparison, the *in vivo* control of ice nucleation in microenvironments, such as exist in living cells or intact organisms, is much better developed and will be discussed in the following sections.

5. *In vivo* ice nucleation and its control

The subject of *in vivo* cold injury has already been referred to, and the fundamental differences between chill and freezing have been emphasized. A further distinction must now be drawn between permanent suboptimal temperature conditions and transient or seasonal fluctuations in temperature. Species that live in permanent sub-zero temperature habitats, e.g. the Arctic oceans, will need to be completely protected against any freezing of their bodily fluids; this process is termed freeze resistance. These species exist in habitats where their bodily fluids are permanently in the undercooled state, i.e. below their equilibrium freezing points. On the other hand, where organisms experience transient cold periods, survival is achieved more economically by a period of acclimatization, during which the organism prepares itself biochemically for the osmotic stress to come. When the stress is removed, e.g. by a rise in temperature, the biochemical changes are reversed and the organism loses its hardiness. The period required for seasonal acclimatization can vary from several months, in the case of trees, to a few minutes, for cold hardy micro-organisms. The fully acclimatized organism will then be able to tolerate partial freezing of selected domains of tissue water (Franks 1985).

It has been found that, in acclimatized organisms, freezing of plasma water can be tolerated, whereas freezing of intracellular (cytoplasmic) water is invariably lethal. The acclimatization process therefore involves a modification of the intracellular aqueous substrate. This can be achieved in different ways, either by a redistribution of water across the plasma membrane or by the synthesis of substances that can modify the nucleation and/or growth behaviour of ice crystals.

Water redistribution in response to extracellular freezing is governed principally by the permeability of the plasma membrane to the osmotic flow of water. If the cooling rate is such that water cannot be removed from the cytoplasm fast enough, the nucleation temperature of ice in the cytosol is likely to be reached, with lethal