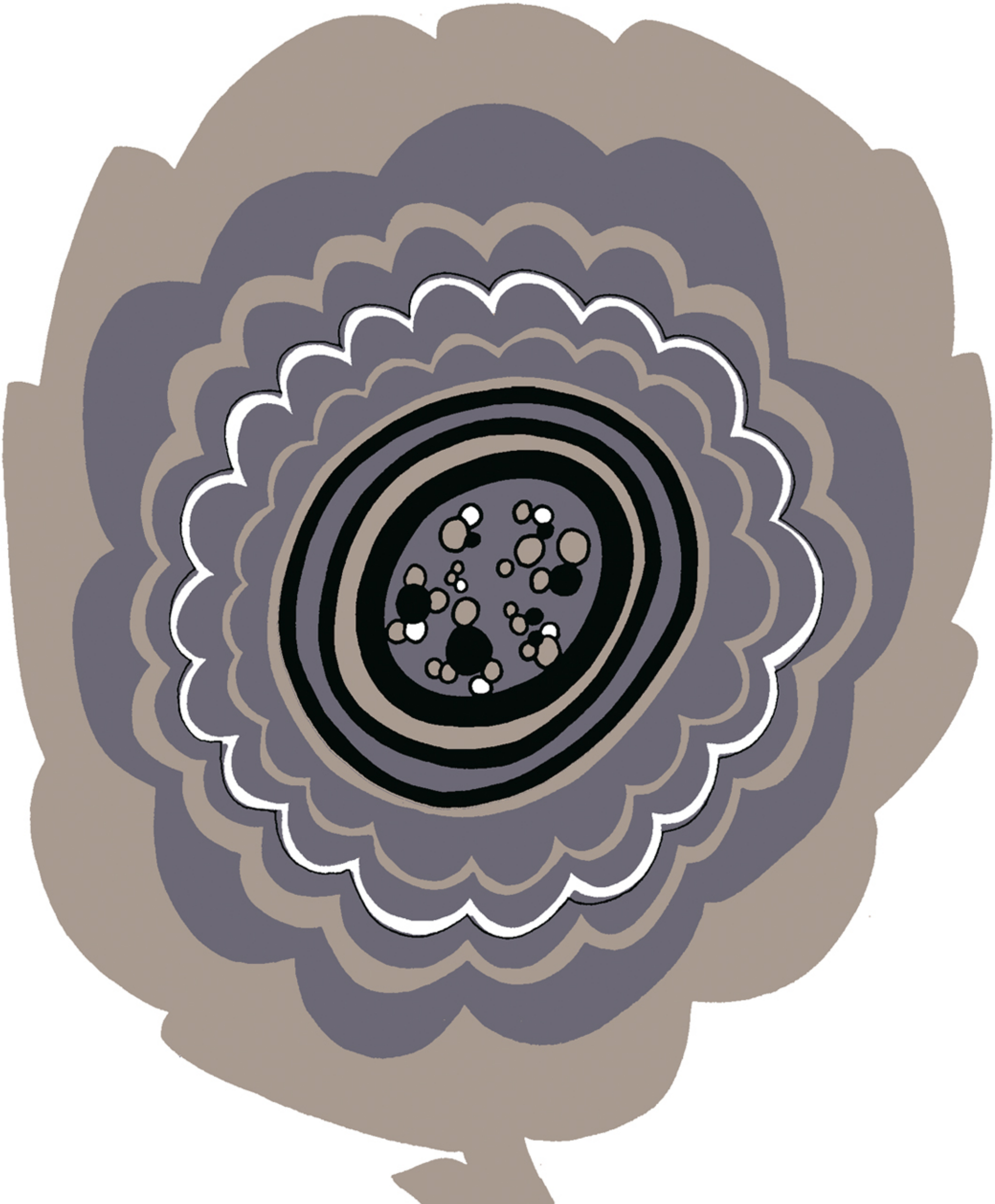
Recent Advances
in Food Science
Volume 4

Low Temperature Biology of Foodstuffs

Edited by
John Hawthorn
Department of
Food Science,
University of
Strathclyde

E. J. Rolfe
National College of
Food Technology,
University of
Reading



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PROFESSOR JOHN HAWTHORN
Department of Food Science,
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and

PROFESSOR E. J. ROLFE

National College of Food Technology University of Reading

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Organizing Committee

DR. E. C. BATE-SMITH, formerly Director, Low Temperature Research Station, Cambridge (Agricultural Research Council).

PROFESSOR JOHN HAWTHORN, Department of Food Science, University of Strathclyde, Glasgow.

DR. M. INGRAM, Director, Meat Research Institute, Langford, near Bristol (Agricultural Research Council).

DR. J. LOVERN, Director, Torry Research Station, Aberdeen, (Ministry of Technology).

PROFESSOR E. O. MORRIS, Department of Applied Microbiology, University of Strathclyde, Glasgow.

PROFESSOR E. J. ROLFE, Principal, National College of Food Technology, (University of Reading), Weybridge.

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This book, with one additional paper, forms the proceedings of a NATO advanced study institute held at the University of Strathclyde in September 1966. The organizers wish to record their indebtedness to the Nort Atlantic Treaty Organization for providing the financial support which made the institute and this book possible.

List of Contributors

PROFESSOR J. CHRISTOPHERSEN, Director, Bakteriologisches Institut der Bundesversuchs- und Forschungsanstalt für Milchwirtschaft, Hermann-Weigmann Strasse 3-11, Kiel, West Germany.

DR. J. J. CONNELL, Principal Scientific Officer, Torry Research Station, P.O. Box 31, 135 Abbey Road, Aberdeen, Scotland.

DR. J. D. DAVIES, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, England.

DR. W. J. DYER, Fisheries Research Board of Canada, Halifax Laboratory, P.O. Box 429, Halifax, Nova Scotia, Canada.

DR. J. C. FIDLER, Ditton Laboratory, (Agricultural Research Council), Larkfield, Maidstone, Kent, England.

DR. D. G. GUADAGNI, Head, Food Appraisal Investigations, Vegetable Laboratory, Western Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture, 800 Buchanan Street, Albany, California 94710, U.S.A.

DR. J. GUTSCHMIDT, Bundesforschungsanstalt für Lebensmittelfrischhaltung, 75 Karlsruhe, Engesserstrasse 20, West Germany.

DR. J. HALLETT, Associate Research Professor, Reno Campus, Desert Research Institute, University of Nevada, Reno, Las Vegas, U.S.A.

DR. J. M. HAYNES, School of Chemistry, University of Bristol, Bristol 8, England.

DR. MARGARET HUDSON, Department of Botany, The Hatherly Biological Laboratories, Prince of Wales Road, Exeter, Devon, England.

DR. D. IDLE, Botany Department, University of Birmingham, P.O. Box 363, Birmingham 15, England.

MOGENS JUL, Director, Danish Meat Research Institute, Maglegardsvej 2, Roskilde, Denmark.

DR. R. A. LAWRIE, Reader in Food Science and Head of Food Science Laboratories, University of Nottingham, Sutton Bonington, Loughborough, Leics., England.

DR. R. M. LOVE, Principal Scientific Officer, Torry Research Station, P.O. Box 31, 135 Abbey Road, Aberdeen, Scotland.

PROFESSOR B. J. LUYET, Director, Department of Biophysics, American Foundation for Biological Research, RFD 1, Box 54 Madison, Wisconsin 53716, U.S.A.

DR. G. MOCQUOT, Station Centrale de Recherche Laitières et de Technologie des Produits Animaux, Jouy-en-Josas, (S. et O.), France.

DR. IRENA MODLIBOWSKA, Pomology Section, East Malling Research Station, Maidstone, Kent, England.

T. N. MORRIS, Formerly of the Low Temperature Research Station, Cambridge, England.

PROFESSOR G. NÉMETHY, The Rockefeller University, New York, New York 10021, U.S.A.

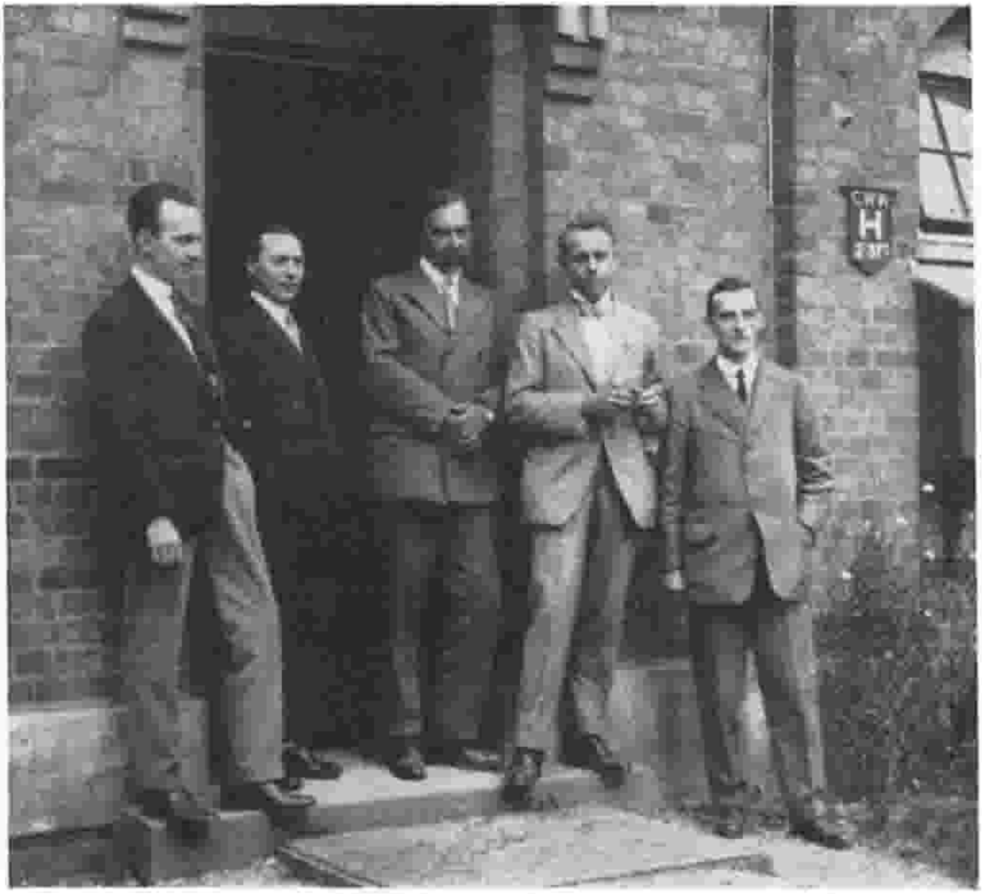
ROBERT L. OLSON, Assistant Director, Western Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture, 800 Buchanan Street, Albany, California 94710, U.S.A.

DR. W. D. POWRIE, Associate Professor of Food Science, Department of Food Science and Industries, College of Agriculture, University of Wisconsin, Babcock Hall, Madison, Wisconsin 53706, U.S.A.

DR. KURT A. SANTARIUS, Institut für Landwirtschaftl. Botanik der Universität Bonn, 53 Bonn, Meckenheimer Allee 176, West Germany.

PROFESSOR J. L. STOKES, Chairman, Department of Bacteriology and Public Health, Washington State University, Pullman, Washington 99163, U.S.A.

L. VAN DEN BERG, Division of Biosciences, National Research Council, Ottawa 2, Canada.



An early Cambridge photograph of founder members of staff of the Low Temperature Research Station. The British contribution to the systematic study of the effects of low temperatures on foodstuffs can be traced directly back to the pioneer work of this group.

Left to right: Dr. T. Moran. C.B.E., D.Sc., Sc.D. (Now Director of Research, British Flour Millers' Research Association); Mr. J. J. Pique who planned the first refrigeration installation; Sir William B. Hardy. LL.D., F.R.S.; Dr. Franklin Kidd, F.R.S. (Director of Food Investigation 1947–57); Dr. C. West, O.B.E. (Superintendent of the Ditton Laboratory, Kent 1931–49).

Foreword

E. C. BATE-SMITH*

THE recognizable beginning of the study of low temperature biology can be seen in the foundation of the Food Investigation Board in 1917, with W. B. Hardy, Biological Secretary of the Royal Society, as Director. The object of the Board was an essentially practical one—to study the preservation of food by cold—and the laboratory it set up, the Low Temperature Station for Research in Biochemistry and Biophysics, had this simple, single objective. Its field of activity was a virgin one, and the yield from it was rich and immediate. The combination of Hardy's knowledge of living tissues and colloid science, Kidd and West's knowledge of plant physiology, Moran's physical chemistry and Piqué's practical know-how not only laid the foundations of low temperature biology and post-harvest physiology, but also those of quick-freezing, deep-freezing and, in due course, freeze-drying.

From these beginnings in the early 1920's sprang not only the work of several associated laboratories—Torry Research Station in Aberdeen and Ditton Laboratory in Kent—but also the Shipowners' Refrigerated Cargo Research Council, the Food Preservation Division of the Australian CISIRO, and the Meat Research Institute of New Zealand. In 1966 the Low Temperature Research Station ceased to exist, its function being divided between two new laboratories, the Food Research Institute at Norwich and the Meat Research Institute at Bristol, but its influence will continue to be represented by the staffs of the laboratories I have named, many members of which either began their careers, or had their early training, in it. The course, of which this book is an outcome, had its origin in and owes its name to this influence, three of the six members of the organizing committee being Directors of the Station or of its associated laboratories, and a fourth owning (by his own admission!) the origin of his distinguished career in Food Science to the stimulus of the Summer Course he attended in Cambridge in 1948.

To many of us the practical application of the knowledge and understanding of the behaviour of foods at temperatures below the ambient will always be embodied in the person of Richard Gane, until his illness

---

* Formerly Director, Low Temperature Research Station, Cambridge. Present address: A.R.C. Institute of Animal Physiology, Babraham, Cambs.

and retirement in 1965, the head of what we used to call the Biological Engineering Section at the Low Temperature Station. It was he who did the pioneering work on freeze-drying just before the war, organized trial shipments of everything from frozen mutton to bananas, and gave immediate and soothing answers to the innumerable and diverse questions on transport and cold storage that kept his telephone busy throughout the working day. In devising the present course this communication between fundamental science and industrial practice has been the dominant consideration, and the organizers feel themselves fortunate in having persuaded contributors to it who combine knowledge of their subject with ability in imparting it. The reason why the experiences of the practitioner are as important as the experiments of the specialist is that it is only by the *written* record of these experiences that the usefulness of the researches on which they are founded can be evaluated. Not only must knowledge be used: it must be *known* to be used, its origin recognized, and—why not?—acclaimed.

As one of the organizers of the course, I feel we have, in fact, achieved what we set out to do—to identify the extent to which scientific knowledge of low temperature biology is being applied in current industrial practice. To me, this is the meaning of technology. It is a stream, but one flowing in two directions, for it is from the experience of industry that science derives the impetus and direction for the next round of its activities.

The Structure of Water and of Aqueous Solutions

GEORGE NÉMETHY

INTRODUCTION

Many of the important chemical properties, including conformational stability and biochemical specificity, of macromolecules and of molecular aggregates in biological systems depend on the interaction of their constituent groups with the surrounding solvent medium. These interactions, in turn, are influenced decisively by the structural features of liquid water and by the structural changes in water, caused by solutes.

On freezing, these interactions are altered. The freezing process itself may be influenced by the presence of solutes. Whilst very little basic information is available on the effect of solute interactions during the freezing of water, an understanding of this process certainly requires an appraisal of the properties of aqueous solutions. In this paper a summary is presented of current views of structural features in water on a molecular level[1,2].

THE STRUCTURE OF PURE WATER

In most liquids composed of small, simple molecules, the molecules are closely packed, i.e. each molecule has nearly the maximum number of nearest neighbors (= coordination number), but there is no spatial order beyond the nearest neighbors. For example, the coordination number for molecules of liquid metals or of rare gases is 10–11 in the liquid state, while it is 12 (the maximum) in the crystalline solid. In water the average coordination number of each molecule is very low in both solid and liquid, and ordering of the molecules in the liquid extends over at least several molecular diameters, due to the formation of intermolecular hydrogen bonds.

Hydrogen bonds result primarily from a strong electrostatic interaction between the hydrogen nucleus attached to an electro-negative atom (usually N, O, or F) as the "donor" and a "nonbonded pair" of electrons or another electro-negative atom, acting as the "hydrogen bond acceptor". However, there is also a contribution to the energy of the hydrogen bond from electronic arrangements similar to those occurring in the formation of covalent chemical bonds[3]. This contribution results in a directional preference: strong hydrogen bonds form when the donor–hydrogen covalent bond and the nonbonded electron pair of the acceptor atom are co-linear. Each water molecule can participate in four hydrogen bonds, acting in two as a donor and in two as an acceptor (*Figure 1*). The construction of spatially extended networks of hydrogen-bonded molecules of water is made possible only by the presence of two donor and two acceptor sites per molecule. This property makes water unique among all simple substances. It has also been shown[3] that it is more favorable energetically if a water molecule forms several hydrogen bonds at once rather than only one.

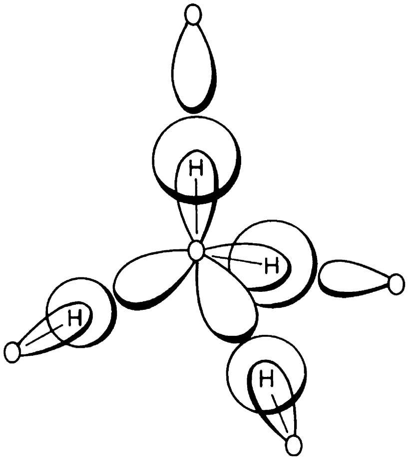


Figure 1. Spatial arrangement of the four hydrogen-bonded neighbours of a water molecule. The neighboring molecules occupy the corners of a regular tetrahedron. The central water molecule acts as donor in two of the hydrogen bonds (top and right) and as an acceptor in the other two. The electron orbitals involved are indicated (not to scale).

This requirement of cooperativeness leads to the concept of the formation of extended regions of hydrogen bonding (= clusters) in the liquid. The theory of water structure, described later, is based on this idea. Before considering this theory, it is profitable to review briefly the structures of the solid forms of water[1].

THE STRUCTURE OF WATER IN THE SOLID STATE

Ice I

This is the normal form at low pressures. In it, the molecules are arranged tetrahedrally and are connected to each other by hydrogen bonds of the "normal" length of 2.76 Å (*Figure 2*). Due to the low coordination number of four, there are relatively large empty spaces between the layers of molecules (*Figure 2*): the density of ice is about half of what it would be if the molecules were close-packed. The hydrogen bonds are straight, and the structure is thus virtually strain-free.

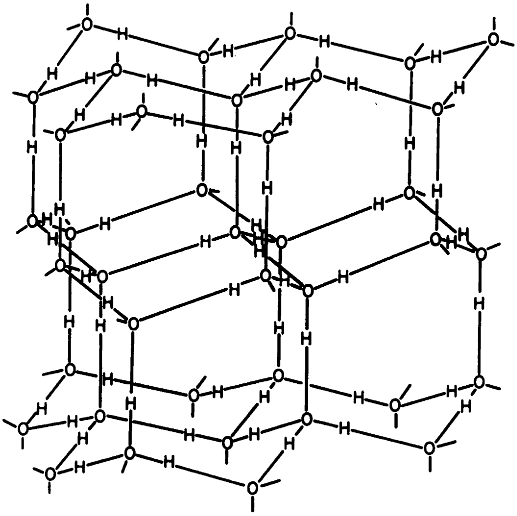


Figure 2. Arrangement of hydrogen-bonded water molecules in ice I.

High-pressure forms of ice

Six different crystalline forms, different from ordinary ice, exist at high pressures (above 2000 atm), each in a certain pressure and temperature range[4,5]. In them, the water molecules are fully hydrogen-bonded, but are arranged so that space is more fully utilized: often two interpenetrating hydrogen-bonded networks are formed[5].

Since the hydrogen bonds are bent or the molecules compressed, such strained structures do not occur under the conditions of interest here.

Cubic ice

This is a metastable form of ice which exists at low temperatures (below − 140°C) but it can only form under special conditions, such as on condensation from the vapour[6]. It cannot be obtained by cooling ordinary ice. Its hydrogen-bonding arrangement is a modification of that of ice I, so that it crystallizes in a cubical form instead of the hexagonal crystal structure. The arrangement of the nearest neighbors and of the empty spaces around a molecule is very similar to that shown in *Figure 2.* On warming it changes over into ice I.

Gas hydrates

These crystals belong to the general class of clathrates, i.e.inclusion compounds, in which one substance is trapped in cavities of molecular size, found in a spatial network formed by the second substance. No chemical bonds are formed between the two substances. In the gas hydrates, the framework consists of water molecules, hydrogen-bonded to each other. Each water molecule forms four hydrogen bonds, but the arrangement of the molecules is more open (*Figure 3*) than that in ice. Approximately spherical cavities form with diameters of 5.2 and 5.9 Å ("class I hydrates") or of 4.8 and 6.9 Å ("class II hydrates")[7]. All or most of the cavities are filled with one molecule each of the second component (or components), which must usually satisfy two conditions:

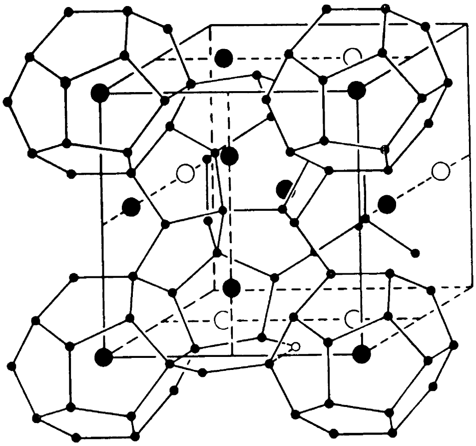


Figure 3. Arrangement of molecules in the crystal of a gas hydrate (class I). Water molecules are represented by small circles, with hydrogen bonds along the heavy lines. Guest molecules occupy the points marked by large circles. (v. Stackelberg and Müller[7]. Reproduced with permission of the *Zeitschrift für Elektrochemie.*)

(a) It must be inert, i.e. it should not physically interact strongly with water molecules. Hydrocarbons, alkyl halides, halogens, argon and the higher rare gases, hydrogen sulphide and its alkyl derivatives, etc. satisfy these conditions. The presence of OH or NH_2 groups prevents the formation of gas hydrates, because of the hydrogen-bonding interaction with neighbouring water molecules.

(b) It must be small enough to fit into the cavities of the structure without significant distortion, i.e. its largest van der Waals' diameter must be < 5.9 or < 6.9 Å for the two classes of hydrates, respectively.

Thus, isobutane or benzene can form gas hydrates (the latter with some distortion), but not n-butane or n-butyl iodide[7].

A list of substances forming gas hydrates and of their physical properties was given by v. Stackelberg and Müller[7]. Some gas hydrates exist only at pressures of the order of 10 atm or higher, but several can form at atmospheric pressure and at temperatures ranging up to +15°C. The largest guest molecules fitting the cavities without distortion form the most stable gas hydrates. The crystals are stabilized by van der Waals' forces acting between the guest molecule and water molecules forming the cavity. Since these forces fall off very rapidly with increasing distance, they make the largest contribution for large guest molecules which have many close contacts with the water molecules. For the same reason, a gas hydrate crystal is only stable when all or most of the cavities are filled with the inert molecules. The gas hydrates crystallize in the cubic form. However, the arrangement of the hydrogen bonds in these crystals and in those of "cubic ice", discussed above, is very different, and the two are incompatible with each other.

Recently, another more complicated class of clathrate-type hydrates has been observed[8], *viz.* those formed by large tetraalkyl ammonium or trialkyl sulfonium salts. In these structures, the cavities are incomplete. The nitrogen or sulfur atom replaces a water molecule of the network at the junction of four cavities. Its alkyl substituents extend into the four (or three) adjacent cavities. The anion, usually F^-, is built into the water network. Here, too, those hydrates are the most stable in which the alkyl side chains just fill the cavities.

No hydrates of H_2, He, and Ne have been observed. These molecules are too small to stabilize the large cavities of the hydrate structures. They might fit into the empty spaces of the ice I structure, but presumably they could readily escape from these empty spaces.

The freezing of water

Among the various forms of ice, only ice I is produced with the freezing of water or of aqueous solutions at low pressures, both on slow and rapid freezing. The only other low pressure form, cubic ice, cannot form from the liquid. Although local molecular arrangements similar to those in gas hydrates are important in aqueous solutions (as discussed below) and presumably also at interfaces with nonpolar substances, gas hydrate type crystals cannot ordinarily form with freezing of solutions. Nonpolar substances may prefer the formation of thin gas-hydrate-like layers of water next to them (see below), but these layers cannot be extended to form the basis of a separate crystal phase because "empty" gas hydrate crystals are unstable.

THE STRUCTURE OF LIQUID WATER

Many anomalous physical properties of liquid water, such as the high heat capacity, the temperature dependence of the density, etc., indicate that some structural order of ice is retained in the liquid. This is ascribed to the presence of hydrogen bonds in the liquid. Some hydrogen bonds break on melting, and the coordination number increases above four. This explains the volume decrease upon melting. As the temperature increases, the extent of order diminishes. At high temperatures and pressures, the behaviour of water approaches that of normal polar liquids.

Within the last five years, several theoretical treatments of liquid water have been presented (reviews can be found in refs. 1 and 2), but the exact nature of its structure is still in dispute. The presence of extensive hydrogen bonding is generally accepted, but there are vast differences in the details of assumptions about the structure.

One of the controversies involves the amount of hydrogen bonding present in the liquid, since there are ambiguities in the interpretations of results by various experimental methods. Estimates vary from 50 to 99% of unbroken hydrogen bonds near the freezing point. In interpreting the properties of water, it should not be forgotten that, even when all hydrogen bonds between adjacent water molecules are broken, electrostatic interactions persist between them. Therefore, even water in such a state (which may exist at high temperatures) would be a highly polar liquid and would not vaporize easily.

Two schools of thought dominate the theoretical interpretation of the properties of water. According to the "continuum theories", the surrounding of each water molecule in the liquid must always be the same as that of any other. This would mean that some kind of distorted hydrogen-bonded arrangement should persist in a uniform manner throughout the liquid[9]. According to the "mixture models", a certain fraction of molecules participate at a given moment in the formation of hydrogen bonded structures, while others have their hydrogen bonds broken[2, 10]. Recently, Frank[10] has summarized the comparison between the two points of view.

On theoretical grounds, the cooperative nature of hydrogen bonding, discussed above, favours a "mixture model"[3,10]. Once a hydrogen bond is formed, it is easier for the water molecules involved to form further bonds. Thus a "cluster" of water molecules, interconnected by a network of hydrogen bonds, is obtained. The reverse process, the disintegration of the network is also cooperative. Both processes are due to local energy fluctuations. When a small amount of energy is lost from a local region, a cluster forms. As soon as collisions of neighboring molecules supply the energy required to break the hydrogen bonds, the cluster breaks up. Thus, water can be described as consisting of a mixture of "flickering clusters"[3] of hydrogen-bonded molecules and less ordered water. Molecules alternate rapidly between the two structures. Because the energy fluctuations are local phenomena, the size of the clusters is likely to be small (estimates of cluster sizes range to less than one hundred molecules[2], or at most a few hundred), and it is temperature dependent.

On the basis of dielectric relaxation and other similar measurements, it has been estimated[10] that the average lifetime of a cluster is of the order of 10^{-11} sec. This is several hundred times the period of a molecular vibration (10^{-14} to 10^{-13} sec). Thus, clusters are definite structural entities. On the other hand, they are very short-lived on the time-scale of physical measurements, and water molecules change roles very rapidly. Therefore, clusters should on no account be considered as "miniature icebergs" floating around in the liquid, and containing water molecules which permanently have different properties from the others. At a given time a certain fraction of the molecules in the liquid is part of some hydrogen-bonded structure, which has larger molar volume and smaller mobility than the average, as reflected by various macroscopic physical properties of water. No regions within the liquid can be considered as permanently frozen, and no molecule is permanently less mobile or less reactive than others: each molecule alternates between being part of a structured cluster and part of the unstructured region of the liquid in turn.

In addition to the theoretical arguments based on the nature of the hydrogen bond, there are other grounds for favouring the "mixture model": the properties of solutions, the rate of proton transport, the low viscosity, etc., are more easily explained in its terms than in those of the continuum theory.

Details of the structure

In most theories, an attempt is made to describe the clusters and the non-hydrogen-bonded liquid as precisely as possible.

The structure of the clusters is usually equated with that of one of the crystalline forms of water, since these structures allow the best hydrogen-bond arrangements. Certain structures can be easily excluded. Thus, clusters at low pressures are not likely to have a structure like any of the high-pressure crystalline forms of ice. Cluster structures similar to the gas hydrates have been proposed as the main component, with the non-hydrogen-bonded molecules occupying the cavities[11]. This is a possibility, and similar structures presumably occur in some solutions (see below), but they, too, seem unlikely to form the main contribution to structured water: the strongly polar water molecule would interfere with the formation of the gas-hydrate cage. Clusters with ice-I-like molecular arrangement can exist free of distortion, and, therefore, probably occur to a large extent.

However, one should not take a narrow point of view about possible cluster structures and should not restrict them to analogues of crystals.

In crystals, the hydrogen-bond arrangement has to be regular because it has to extend indefinitely in all directions. No such necessity arises in the clusters, particularly when they are small. They can be composed of irregular networks of hydrogen bonds. Such irregular networks cannot, of course, be extended beyond a few dozen molecules without disruption of many hydrogen bonds. However, for small clusters, this is no disadvantage. It is even in better accord with molecular dynamics, since not all water molecules may reorient fast enough during the formation of a cluster to allow the formation of a regular structure. If the majority of the clusters in the liquid is postulated to have an irregular structure, this also explains while supercooling of water occurs easily: such clusters are not good nuclei for crystallization.

The description of the non-hydrogen-bonded portion of the liquid is even less definite. The molecules in this class have been thought to exist within the gas-hydrate-like cages[11], as discussed above, or to fill the space between the clusters, packing like other polar liquids[2,3]. The latter concept requires the least unusual assumptions. Due to the large dipole moment of water, there is a strong attraction between the molecules even in this region, just like in other, strongly polar liquids, and the motion of the molecules is somewhat restricted. It has been estimated that only about one quarter[2] or slightly more of the heat of sublimation of ice comes from the breaking of hydrogen bonds, and the rest from non-hydrogen-bonding interactions in the liquid.

The qualitative description of water as a mixture of two states, one less dense and highly hydrogen-bonded, the other having higher energy due to the breaking of hydrogen bonds and being packed more densely and more irregularly, can explain many observed properties. For example, while both clusters and the non-hydrogen-bonded liquid expand with rising temperature, the amount of water in clusters decreases gradually. The latter process is accompanied by a volume decrease. The net change in volume with temperature is the resultant of these two tendencies. The minimum in molar volume (at 4°C in H_2O and 11.2°C in D_2O at 1 atm. pressure, and at lower temperatures for higher pressures) occurs where the two tendencies happen to cancel.

Several "two-state theories" of water structure[1] have been based on this idea of two types of structures in equilibrium. However, in a more refined treatment, as needed, for example, for aqueous solutions, this description is inadequate. The small size of the clusters and the resulting surface effect must be taken into account explicitly. While all molecules of a cluster have a restricted mobility and contribute to the high molar volume, those on the surface of the clusters have less than four hydrogen bonds and differ also in some other properties from the molecules inside the clusters. In a small cluster, a large fraction of the molecules are on its surface.

This concept was taken into account explicitly in the theoretical treatment by Némethy and Scheraga[2,12]. In this theory five kinds of water molecules are distinguished, with different energies according to the number of hydrogen bonds they form. Molecules with four hydrogen bonds occur inside the clusters, those with no hydrogen bonds occupy the space between the clusters, and the other three kinds are on the surfaces of clusters (*Figure 4*). It is not necessary to postulate any particular structure for the clusters, as long as approximately the maximum possible number of hydrogen bonds is formed in them. This model gives a quantitative explanation of many physical properties of both ordinary and heavy water and of aqueous solutions. The theory predicts average cluster sizes in H_2O ranging from 91 at the freezing point to 25 near 70°C (the limit of the calculations)[2], with a similar range in D_2O (117 at +4°C, the freezing point, and 27 at 65°C)[12]. The fraction of hydrogen bonds is 0.53 and 0.55 at the respective freezing points, and falls as the temperature increases (*Figure 5*). The theory treats the five kinds of water molecules as independent species with sharply defined properties. This was a mathematical approximation (not affecting essential features of the theory), because distinctions between molecules in liquids are much less sharp.

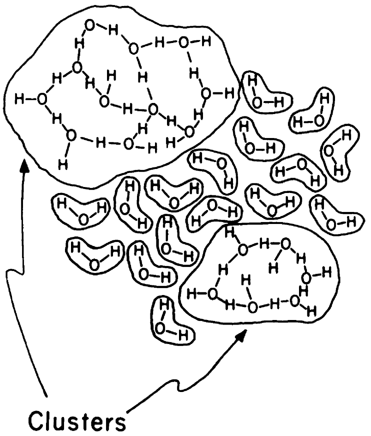


Figure 4. Schematic representation of the structure of liquid water, according to Némethy and Scheraga[2]. Hydrogen-bonded clusters and non-hydrogen-bonded water between the clusters are shown. (Reproduced with the permission of the American Institute of Physics.)

In a recent modification of the theory[13], some details of the physical model are being altered, together with changes in some of the assumptions of the mathematical evaluation of the model. This should lead to improvements in the application to aqueous solutions.