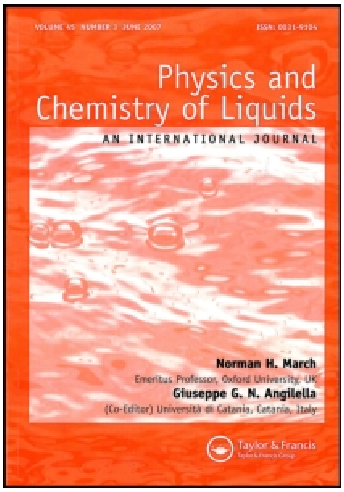

**Physics and Chemistry of Liquids**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713646857

# Water at Biological Interfaces - Structural and Functional Aspects

W. Drost-Hansen[a]
[a] Department of Chemistry, University of Miami, Coral Gables, Florida, USA

**To cite this Article** Drost-Hansen, W.(1978) 'Water at Biological Interfaces - Structural and Functional Aspects', Physics and Chemistry of Liquids, 7: 3, 243 — 348

**To link to this Article: DOI:** 10.1080/00319107808084734

**URL:** http://dx.doi.org/10.1080/00319107808084734

*Phys. Chem. Liq.*, 1978, Vol. 7, pp. 243–348

Printed in Holland

# Water at Biological Interfaces – Structural and Functional Aspects†

W. DROST-HANSEN

*Department of Chemistry, University of Miami, Coral Gables, Florida, USA*

The structure of water near interfaces is reviewed in this paper and evidence presented for the existence of notable changes in the properties of such vicinal water. The depth of the changed surface zone is discussed; it appears that this depth may approach a tenth of a micron. Possible types of vicinal water structures include the high-pressure ice polymorphs and clathrate, cage-like entities. Some examples of thermal anomalies in the properties of vicinal water are reviewed; at least four different thermal anomalies occur between the freezing and boiling point. It is concluded that these anomalies likely reflect higher order phase transitions in the structured units of the vicinal water. These transitions appear not to be notably affected by the detailed chemical nature of the solid substrates with which the ater is in contact. This is referred to as the "paradoxical effect" and is tentatively interpreted in terms of an energy delocalization phenomenon. The vicinal water structures near solid interfaces appear characterized by low energetics and long-range ordering; these vicinal structures may represent the "anti-crystalline" state discussed by Ubbelohde. The importance of such structures in biological systems is examined. Examples are shown of thermal anomalies in the properties of cellular systems, and the role of water in cell physiology is reviewed, including aspects of water/macromolecule interactions, gene action, mechanism of thermal death, and active transport, etc. Non-equilibrium aspects of biological systems are considered briefly in terms of the "dissipative structures" discussed by Glansdorff and Prigogine, and the suggestion is made that the domains of local ordering which may occur as the result of conditions very far from equilibrium may specifically and uniquely involve the intracellular structurally modified water.

## CONTENTS

---

† Presented at XV Solvay Conference on "Electrostatic Interactions and the Structure of Water," Brussels, June 1972.

Contribution No. 6, LABORATORY FOR WATER RESEARCH

## I INTRODUCTION

The structure of bulk water continues to escape a satisfactory description. A large number of models are currently in existence, ranging from the "average, continuum" model to mixture models with vastly different, structured entities proposed for the low density species. Ample reviews of water structure have been presented in this Conference and elsewhere (see, for instance, Kavanau (1964); Frank (1970, Eisenberg and Kauzmann (1969), or the volume edited by Horne (1972)): See "Note added in proof".

The purpose of the present article is to call attention to the probable changes in the structure of water near solid/water interfaces; particularly the type of changes occurring at the interfaces encountered in biological systems. Two major questions will be considered first: what types of structures occur near an interface, and what is the depth of such structural rearrangements? Finally, a brief review is presented of the role which structurally modified water may play in living systems. Notwithstanding the fact that the structure of bulk water itself remains unsolved, it appears that some insight can presently be obtained into the nature (and extent) of vicinal water—including the water of interest in cellular physiology.

## II STRUCTURE OF BULK WATER; COOPERATIVITY OF HYDROGEN BONDING

In view of the availability of many reviews of water structure, only one point will be emphasized in this section, namely the likely cooperative nature of hydrogen bonding in water.

The notion of cooperativity in the hydrogen bonding in water is due to Frank (1958), who suggested that two neighboring water molecules, when forming a hydrogen bond between them, essentially undergo an acid-base interaction in the *Lewis* sense with the result that one molecule becomes more acidic while the other more basic than if the two water molecules remained unbonded monomers. This initial step leads to greater ease for forming additional hydrogen bonding—one acidically, the other by a base mechanism—and this, in turn, results in a "positive feedback." The process is not confined to only "linear" propagation of hydrogen bonding, but may indeed form in other directions and hence promote hydrogen bonding in three dimensions. As a result, Frank states that this will "produce a cooperative character in the whole phenomenon." Frank goes on to say "as a result of this, one expects that, when one hydrogen bond forms in liquid water, a number form and there comes into existence an ordered, or structured cluster of a size and shape determined in part, by the magnitude and spatial distribution of the energy fluctuation which permits the 'latent heat of freezing' to be carried away."

Indications of cooperativity in hydrogen bond formation have been obtained from purely theoretical studies. Thus, Del Bene and Pople (1969), Del Bene (1971), Minton (1971), and others have performed quantum mechanical calculations on hydrogen bond energies, treating, in a quantitative fashion, the total interaction of all nuclei and electrons between small numbers of water molecules. The results, for instance of Del Bene and Pople (1969) clearly indicate that stabilization occurs with ring formation for, perhaps 4, 5, or 6 water molecules (relative to the same number of molecules unbonded to each other). The calculations command the greatest of respect, being truly heroic exploitations of contemporary quantum mechanics by means of available computer technology. Unfortunately, the results, of necessity, still refer to groupings of water molecules in a vacuum rather than in the complex, unknown, fluctuating force fields on the liquid.

However, the results do seem to establish that cooperative bonding is indeed theoretically possible.

Among current theoretical treatments of the liquid, attention will essentially be restricted to the recent study by Perram and Levine (1971). These authors have studied the nature of cooperative hydrogen bonding in water and specifically, the statistical consequences for the existence of flickering clusters. The paper is an extension of two previous studies aimed at solving the problem of accounting for hydrogen bond configurations in water in terms of a theory of cooperative phenomena. The basic approach is to assume that the binding energy for a particular hydrogen bond will increase with the number of such bonds formed between the two molecules under consideration; the increase is supposed merely to be linear in order to make the model mathematically manageable. The model then attempts to describe water as a system of N molecules on a regular lattice of N sites, each with a coordination number of four. From a consideration of the proper weighting factor, and the $2N$ pairs of nearest neighbor interaction energies, a configurational partition function is derived. The resulting equation (Eq. 15, loco cit.) is similar to the partition function for an Ising model ferromagnet, which under suitable conditions, may display order-disorder transitions. It is hardly a surprise that the transition (should such indeed take place according to this equation) is identified with the simple melting of the ice lattice. From a comparison with the Lee–Yang treatment of a lattice gas, Perram and Levine conclude "on the other hand, below the transition, as the temperature of the liquid is increased, it is the clusters of vacancies which grow in size. Carrying this further analogy over to the hydrogen bond problem and taking the reasonable view that the dense bond state is analogous to the lattice liquid above, we infer that as the temperature of the system is lowered, it is the clusters of vacant bond sites which grow smaller, rather than the clusters of bonds which grow bigger." The authors conclude by noting that "the type of cooperative bonding examined here does not imply the existence of flickering clusters or densely bonded regions in liquid water, but instead, the existence of regions containing few bonds, immersed in an extensive network."

The present author does not necessarily subscribe to all the conclusions drawn by Perram and Levine, although it must be admitted that they have made carefully guarded statements regarding the general validity of the theory they have derived. On the other hand, the attempt deserves recognition, because of its very fundamental and even heroic nature. It should be noted finally that the degree of complexity of the treatment and the tenuous nature of the conclusions which can be drawn therefrom, make it probable that an extension of the theory to structured water near an interface is likely decades away.

A recent but far more elementary treatment of the structure of water in terms of an order-disorder model has been given by Arakawa and Sasaki (1970), who based their approach on a two-state model for water to which is applied a Bragg–Williams order-disorder treatment. The water molecules are divided into alpha and beta-sites. If the molecules are completely tetrahedrally bonded, they represent beta sites; if non-hydrogen bonded, alpha sites. On this basis, a partition function is derived and calculated values for the thermodynamic parameters compared with experimental data. No doubt, the theory is again a serious oversimplification, but it does represent an attempt at a statistical mechanical treatment of an order-disorder phenomenon. The reason for stressing such statistical mechanical approaches in terms of order-disorder phenomena will become evident in later sections.

## III WATER NEAR INTERFACES

Over the past fifty years, a number of papers have been published suggesting evidence for enhanced structuring of water over extensive distances near solid interfaces. A number of the claims made in these papers have not stood up to close scrutiny, but a handful of examples remain which appear difficult—or impossible—to "explain away." Some specific examples of this type will be discussed below. A number of review articles on this topic have been presented, notably by Henniker (1949), Low (1961) and Drost-Hansen (1965, 1969).

### A "Classical approach"

Prior to approximately 1940, speculations about unusual properties of water near interfaces in general and especially in biological systems were fairly common. Particularly frequent were speculations regarding "bound water" in biological systems. However, with the advent of the Derjaguin–Landau–Verwey–Overbeek theory of colloidal stability, the field of study of water near interfaces suffered a serious setback†. The impressive semi-quantitative success of the DLVO theory—particularly in the hands of Verwey and Overbeek—without recourse to specific reference to the nature of the water near the interface of colloidal particles, resulted in a general tendency to more or less completely ignore the problem of specific water structures. Hence, a "classical approach" now developed, boldly ignoring structural details of water near interfaces, particularly near charged interfaces. Water was assumed to possess its bulk structure up to the very interface or, at

---

† Except for the continued contributions by Derjaguin.

worst (or best), to within one or two molecular diameters from the solid-water interfaces. Typically, serious studies of structural aspects of water near interfaces became unpopular and the papers addressing themselves to this problem were, on the whole, ignored—a fate worse than rational and severe criticism.

## B Evidence for vicinal structuring of water

### *1 Vapor pressure measurements in narrow capillaries*

Over a period of many years, Shereshefsky has published papers on vapor pressure of liquids in small capillaries. For water, Shereshefsky and Carter (1950) concluded that in cone-shaped capillaries (ranging from 3 to 10 microns in radius) the vapor pressure lowering exceeded the calculated value (based on the Kelvin equation) by factors ranging from 7 to 80. The observed lowering of the vapor pressure is thus extraordinary. Shereshefsky and Carter suggested that this observation is a direct manifestation of long range interactions between the glass wall and the water. The results obtained by Shereshefsky have, to the best of the present author's knowledge, never been seriously questioned! Yet, the results have, with a few notable exceptions, been completely ignored until recent years.

### *2 Derjaguin's viscosity studies*

Derjaguin has extensively studied the properties of liquids to solid surfaces. Among the methods employed has been a dynamic approach, utilizing an oscillating bob viscometer (an optically flat quartz surface and slightly curved surface [the oscillating bob] in close proximity, immersed in water). Very high values were observed for the viscosity of the water for small separations of the surfaces and Derjaguin concluded that structural effects of the solid on the water extended over distances of nearly $\frac{3}{4}$ of a micron. The studies by Derjaguin attracted considerably more attention than the studies by Shereshefsky and co-workers. Derjaguin's experimental approach was, however, open to criticism on grounds of possible spurious effects due to "dust particles," "surface swelling," and/or "surface rugosity."

### *3 Melting point and vapor pressure of water between glass plates*

Hori (1960) studied the vapor pressure of water between glass plates and between quartz plates. For separations between the plates of approximately 0.15 and 0.10 microns (or less), the water between glass plates and quartz plates possessed a nearly temperature independent vapor pressure! Water films between glass plates separated 0.13 $\mu$ did not evaporate in a vacuum (unspecified pressure) at 300°C. Similarly, for thicknesses larger than approximately 10 microns, the water exhibited a "normal" freezing temperature of somewhere between −20° and (slightly below) 0°C—the expected result from supercooling. However, for thicknesses less than 10 microns, the water between the glass plates did not freeze at temperatures below −70° to −100°. These observations also suggested that the surfaces exerted notable effects on the properties of the water in thin films—hence likely revealing structural changes. Hori's experiments have not been seriously challenged.

## 4 *Dielectric studies*

*a Palmer and co-workers* Palmer (1952) and Palmer *et al.* (see Cownie and Palmer, 1952) have studied the dielectric properties of wet clays. Palmer specifilly addressed himself to the change which would be expected as the amount of water adjacent to the solid was increased. Thus, the initial adsorbed water on the clay surface might possess the properties of "bound water" with presumably the same properties as those of ordinary ice. As additional amounts of water are added, more nearly bulk-like properties would prevail. It is important to note that Palmer suggested that the change from structured and unusual, bound water to ordinary water might be exponential. Palmer concluded that the "bound" water in the adsorbed film, vicinal to the clay surface, possesses a dielectric constant far less than that for ordinary bulk water. Indeed, a dielectric constant of three (as compared to eighty) for the water in clay pastes was deduced from these studies at radio frequencies (60 MHz to about 9,000 MHz). Another study by Palmer *et al.* (1952) also demonstrated unusual dielectric properties of water between mica plates (measured at 2 and 3 MHz). Perhaps this is one of the most remarkable of Palmer's observations, matched only by the more recent measurements by Metsik and Aidanova. Palmer and co-workers observed that the dielectric constant of water films was as low as 20 for films with a thickness of 5 microns. This value decreased to 10 for films about 2 microns in thickness. An interesting note in this connection was made by Palmer and co-workers, namely that "it would appear that the water film is tending to act not as 'solid' water but rather as 'liquid ice'."

For a survey of dielectric properties of water adjacent to interfaces, see the monograph by McIntosh (1966). Attention is also called to the studies by Ebert (1964, 1971), by Muir (1954), Goldsmith and Muir (1960) and by Windle and Shaw (1956). Johnson and Neale (1963) conclude from a study of water adjacent to alumina, carborundum and other solids, that the "orienting effect of the surface extends for a considerable distance through the continuous medium." Again, this study appears also to have been generally ignored.

*b Studies by Zhilenkov* Derjaguin refers to the dielectric studies by Zhilenkov (1963) in which it was found that the dielectric constant for water on silica gels is only somewhere around 8 to 10. This compares favorably with the conclusion by Palmer. Furthermore, this value appears unchanged upon further addition of water up to as many as 10 to 20 layers. Again, this resembles qualitatively the conclusions reached by Johnson and Neale. Later, Nekrasova, Zhilenkov and Federov (1968) observed that water adjacent to silica gel appeared to have a special structure differing from that of bulk water.

*c Other studies on the water/mica interface system* Afanas'ev, Metsik and Popova (1966a,b) have studied the dielectric properties of water adjacent to mica surfaces. These authors also observed highly unusual behavior for the dielectric constant of water in this system. Thus, it was found that the depth of the layers affected were several hundred times the depth of a monomolecular layer of water (approximately 0.1 microns in thickness). We later return to the studies by Afanas'ev, Metsik and co-workers.

Ruthberg and Frenkel (1964) extended the studies of the dielectric properties of water adsorbed on mica surfaces. These studies were essentially low frequency studies (100 to 50 kHz). From this rather careful investigation of of dielectric properties of freshly cleaved mica as a function of relative humidity (from which the water was adsorbed), it was concluded that the films of water adsorbed from the vapor phase in this case might not be uniform but rather be localized in patches, presumably surrounding "high energy sites."

### 5 *Drainage experiments*

In the preceding section, we dealt primarily with two different types of systems: those essentially consisting of two "half-infinite spaces," of solid and water, respectively, and three-phase systems of the type solid/water/air for very small amounts of water (i.e., exceedingly "thin layers"). Some "drainage" experiments by Tschapek and Natale deserve particular attention, as these attempt to bridge the gap between the two classes of systems. Thus, in the experiments by Tschapek and Natale (1966; 1969), initially water-filled glass tubes are suddenly emptied of bulk phase water (or aqueous solution) and the thinning of the draining film is monitored through conductances (or potential) measurements. The essence of the results obtained by Tschapek and Natale are summarized in Figures 1 and 2. In the first illustration is shown a typical example of the variation in apparent thickness (as judged from surface conductance) as a function of time. It appears that in a free draining film, a nearly stable, limiting value for the

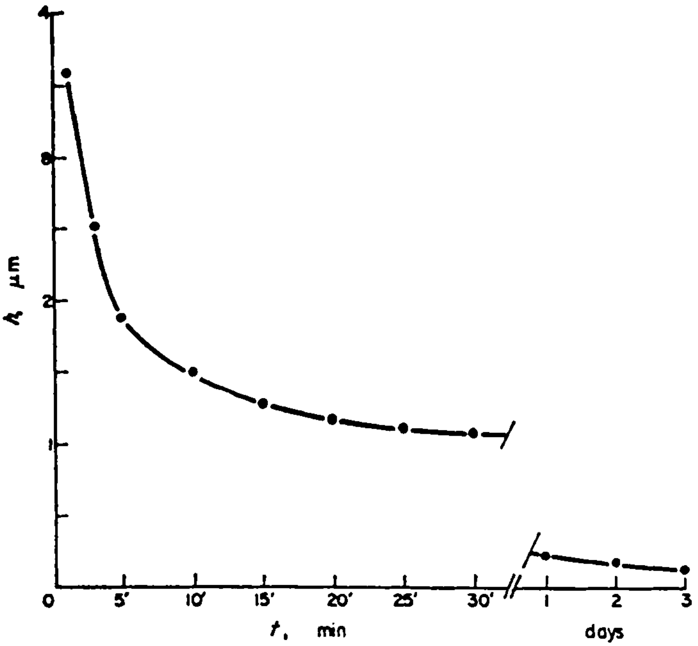

FIGURE 1 Apparent water film thickness on walls of free draining quartz tube as a function of time of drainage. (Tschapek, Santamaria and Natale, 1969).

thickness of the water adjacent to the interface is achieved after approximately 24 to 48 hours. This thickness is of the order of 0.07 microns. Notable changes, however, most likely occur in the conduction mechanism in such films, as illustrated in Figure 2 which shows the variations in specific conductivity as a function of apparent film thickness.

### 6 *Heat conductivity experiments*

Frontas'ev (1956) has measured the heat conductivity of what was prepresumably bulk water, as a function of temperature. This study resulted in a highly anomalous temperature dependence as discussed by the present author (1967). It appears now that the interpretation then given by both Frontas'ev as well as the present author may require some modification. The anomaly observed around 30 (to 40′) was interpreted as suggesting a change in the structure of water (the bulk water). In view of later developments (see particularly Drost-Hansen, 1969, and Section IV.D.I), it appears likely that all thermal anomalies in aqueous properties are manifestations of surface phenomena. Thus, it is suspected that the measurements by Frontas'ev

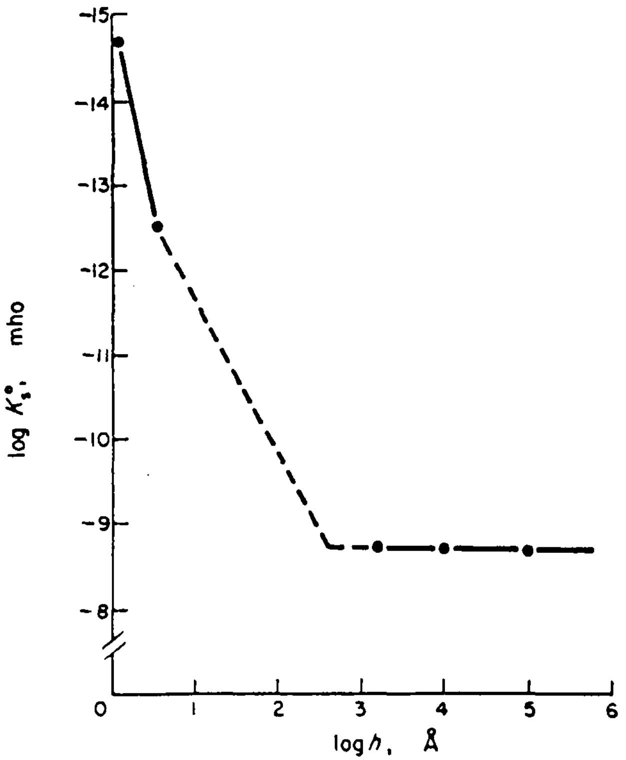

FIGURE 2 Specific surface conductance of water on flat quartz surface as a function of film thickness. (Tschapek, Santamaria and Natale, 1969).

exhibited systematic trends due to surface effects, giving rise to the thermal anomaly.

More recently, Metsik and Aidanova (1966) have carefully measured the thermal conductivity of water between mica plates. The results are of considerable interest. The thermal conductivity was measured for water contained between successive sheets of mica (each mica sheet 20–30 microns thick, with an area of 20 sq cm). Water was admitted to the spaces between adjacent mica surfaces and the entire assembly could be compressed to varying degrees by hydraulic pressure or in a vice. The results of this study were anomalously high conductivities for the vicinal water. The thermal conductivity of 0.1 micron thick films of water was demonstrated to be more than an order of magnitude higher than the thermal conductivity of bulk water (in fact, 70 times larger). This anomalously high conductivity is particularly interesting in that it exceeds the thermal conductivity of the completely ordered lattice of water in the form of crystalline ice!

### 7 *Anomalous viscosity data*

Viscosity measurements have played a considerable role in suggesting structural changes of water near interfaces. However, these experiments have often rightly been criticized due to the possibility of spurious effects, for instance, contamination with dust particles. Earlier, viscosity date obtained by Derjaguin were discussed in III.B.2; in the present section some results by Peschel and Adlfinger (1970) will be discussed. These authors have constructed a device with which it is possible to measure accurately (and with great sentivity) the forces between solid surfaces in close proximity. The instrument consists essentially of two quartz plates—one perfectly flat with an extremely high polish; the other with equally high polish but with a slight curvature (corresponding to spherical surface with radius approximately one meter). By determining the rate of approach as the hemispherically shaped to plate settles onto the flat surface, "squeezing" water out of the space between the plates, the apparent viscosity of the water between the plates can be calculated. Figure 3 shows some of the results obtained by Peschel and Adlfinger. Note the high values of the "surface viscosities" observed in this case, and particularly the depth over which such anomalous viscosity values are observed. The range of changed viscosity certainly exceeds a tenth of a micron. We return to studies using essentially the same experimental approach in the section on disjoining pressure (IV.B.1).

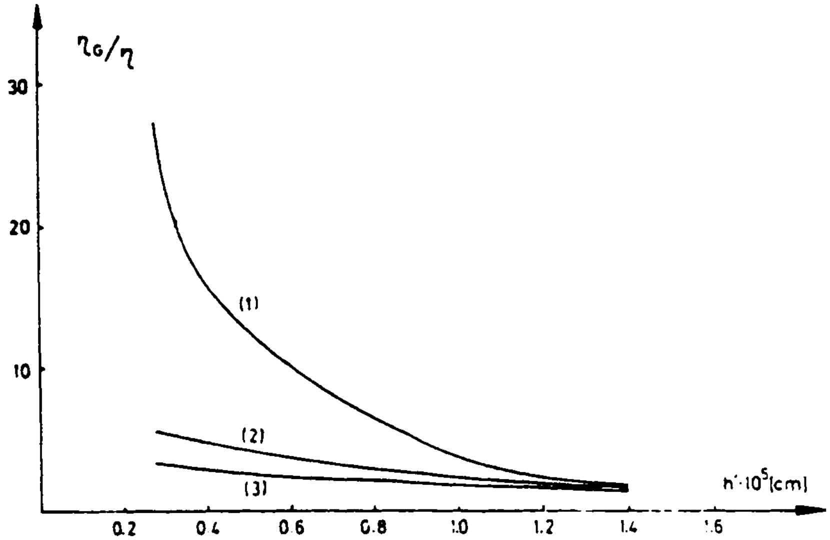

FIGURE 3 Relative viscosity of water (at 20°C) between quartz plates for various plate separations (h'). (Peschel and Adlfinger, 1970).

### *8 Dielectric studies of bimolecular leaflet membrane*

The general topic of dielectric properties of water in and adjacent to membranes is somewhat outside the topic of immediate interest here; however, the measurements by Coster and Simons (1970) deserve mention. Using a fairly simple low frequency capacitance bridge, dielectric measurements were made on a bimolecular lipid membrane (water on both sides of the membrane). The treatment is subject to a number of assumptions; however, it appears that the dielectric properties of the water adjacent to the membrane are highly anomalous. Depending upon the assumptions made in the calculations, the modified, vicinal water layers may extend anywhere from a tenth of a micron to as much as four microns from the interface! Work has been initiated in this laboroatory to attempt to check (and possibly extend) the results by Coster and Simons.

## C Nature of stabilized water structures at interfaces

### *1 The three basic questions*

If the nature of water near interfaces does indeed differ from the structure (or structures—in the mixture model sense) of bulk water, there are essentially three major questions to be answered:

a) what type of water structures are possible?

b) over what depths do these structures persist?

c) how do these structures (and the depth) depend on the chemical nature of the interface to which the water is adjacent?

In this section, a brief outline of possible types of structured water is discussed and the possible depth of such orientations. However, for reasons which will subsequently become clear (IV.C.1), a discussion of the specific role of the chemical nature of the solid will be postponed until later.

### *2 Possible types of structured water elements*

*a High pressure ice polymorphs* At least 9 polymorphic forms of ice exist. Thus, the phase diagram of water is exceedingly complex. The stable forms of ice are Ice-Ih, II, III, V, VI, VII, VIII, and IX. In addition, Ice-IV occurs but is metastable with respect to Ice-V. Vitreous and cubic ice (Ice-Ic) are known—the vitreous form possibly being identical to glassy water. The cubic ice is formed from vitreous ice by increasing the temperature. Further increase in temperature transforms Ice-Ic (irreversibly) to Ice-Ih (with a small change in enthalpy).

To achieve bulk phases of the high pressure ice polymorphs, extreme pressures are often required. Thus, at room temperature, water is in equilibrium with Ice-VI for pressures above approximately eleven kilobars. At most any temperature, pressures in excess of 20 kilobars are required to produce Ice-VII and Ice-VIII. Note that at about 22 kilobars, Ice-VII is stable up to approximately 80°C while the melting point is around 440° at 200 kilobars (see Fletcher, 1970). However, notwithstanding the very high pressures necessary to create the different ice polymorphs, these different structural arrangements may occur more frequently than expected. The thermodynamics of the ice/ice transformations are shown in Table III, from the Monograph by Eisenberg and Kauzmann (1969). Notice that the lattice energy differences rarely exceed 500 calories per gram mole (Ice-VI compared to Ice-VII) and more often differ only between different polymorphs by a few hundred calories per gram mole. Compare this to thermal energy, RT, at room temperature, of about 600 calories. Note further the remarkably small energy difference between Ice-I and Ice-II: only 19 calories per gram mole, or 1/30 of RT. Thus, while thermodynamically unstable, these high-pressure ice polymorph structures may possibly form where (and when) kinetically favored. Thus, Ice-II, for instance, has been postulated as a possible metastable, transient state in the freezing of ordinary water at moderately low rates of freezing (see Drost-Hansen, 1967, and Bogan and Drost-Hansen, 1972).

*b Clathrate structures* Clathrate compounds are essentially solid lattice frameworks of water with a guest species occuping all (or the majority) of the larger, vacant sites in the lattice. Excellent reviews of clathrates have been presented by a number of authors (Jeffrey and McMullan, 1967), Jeffrey (1969), van der Waals and Platteeuw (1959), and Gawalek (1969). From the survey by Jeffrey (1969), we quote briefly, two aspects of clathrate hydration formation of organic compounds in tabular form.

The unique ability of water to form hydrates with various organic molecules in the crystalline state is shown in Table II, also from the article by Jeffrey. Particularly interesting are the organic amines for which polyhedra are reported with hydrogen bonded water molecules in the corners of polyhedra, where the number of $n$ in the $n$-hedra, $(H_2O)_n$, may take on the values of 8, 11, 12, 14, 15, 16, 17, 18, and 26. Note that practically all of these polyhedra are regular polyhedra. Thus, an examination of known hydrate structures strongly suggest that it is possible to make rather "dilute" solid lattice frameworks of water with only a small assist from solute molecules.

*c Some structural details of vicinal water* Only a limited number of specific models have been proposed for structured entities of water near

TABLE I

Classification of organic hydrates[a]

| | Structural characteristics |
|---|---|
| Ices<br>Hexagonal, cubic, high-pressure ices, II–VII[b] | Infinite three-dimensional hydrogen-bonded framework structures |
| Clathrate hydrates: gas hydrates | Nonbonded guest molecules stabilize clathrate voids in polyhedral host structures of hydrogen-bonded water molecules |
| Clathrate hydrates: peralkylammonium salt hydrates | Water and anions form closely related hydrogen-bonded host structures and the cations occupy the voids |
| Semiclathrate hydrates | Water host structure has definite polyhdral clathrate voids occupied by hydrogen-bonded alkylamine molecules |
| Water framework structures<br>Three-dimensional<br>Two-dimensional<br>One-dimensional | Water molecules form hydrogen-bonded nets, sheets, columns, ribbons, or chains. There are no recognizable clathrate voids, and the functional groups of the molecules strongly influence the structure. |
| Hydrates with isolated water molecules | Structure determined principally by the ionic coordination or hydrogen-bonding function of the solute species |

[a] Hydrates ordered in the degree to which $(OH_2)_n$ is structure determining.

[b] B. Kamb, *Science*, **150**, 205 (1965).

TABLE II

Hydration properties of organic molecules in the crystalline state

| | | |
|---|---|---|
| Hydrogen bonding<br>↕<br>Hydrophobic clathration | Carbohydrates, polyols, amino acids, peptides, pyrimidines, purines, urea, ureides, acids and salts, amides, nitriles, aldehydes, phenols, *quaternary methylammonium ion*, glycols, polyoxymethylenes, alcohols, ketones, cyclic amines, aliphatic amines, cyclic ethers, quaternary *n*-butyl- and iso-amyl-ammonium ions, mercaptans, alkanes, alkyl halides (rare gases, halogens, $ClO_2$, $SO_2$, $H_2S$, $N_2O$, $CO_2$, $CS_2$, COS) | Low hydrates<br>↕<br>High hydrates |

interfaces. Earlier, the present author (1969) suggested that at the solid/water interface—where the solid is either ionic or highly dipolar—a layer of oriented water molecules may exist, determined by ion/dipole or dipole/dipole interactions. However, it was felt that the number of these layers would decrease rapidly with distance from the solid, thus likely giving rise to no more than, roughly 2 to 5 molecular diameters of "oriented" water. Adjacent to a non-polar surface, it was proposed that a possible stabilization may occur in the form of a clathrate type hydrate (recall, for instance, that

Pauling (1959) has suggested a structure of water in which the water is the "guest molecule" in its own clathrate hydrate lattice). For non-polar materials with no readily accessible "branches" or side groups (side chains) it was suggested that this stabilization would likely be limited. It was also suggested that if the bulk water possessed a structure which was not "compatible" with the vicinal ordered structures, an intermediary zone of enhanced disorder might exist. This zone, then, would be a region of "lattice mismatch" between the strongly oriented, tightly bonded water adjacent to the interface and the differently structured (but unknown) bulk water structure. In view of the apparent long ranges of orientations which have been reported by many authors, it seems that the above picture is in need of modification. The reasons for challenging the idea of the "three layer model" are discussed also in the section on the "paradoxial effect" (IV.C.). However, it is worth noting that the three layer model discussed above bears some resemblence to the model of ion hydration proposed by Frank and Wen (1957) in which the water molecules in most intimate contact with the ion are oriented due to the strong Coulomb interaction. Between these oriented water molecules in the centrosymmetric field of the ions and the bulk structure may exist a disordered zone.

### 3 *Depth of vicinal orientation*

Inspection of the papers referred to in this section (as well as additional papers; see Drost-Hansen, 1969) suggests that notable structural orientations may be encountered over distances of the order of 0.1 microns adjacent to many solid interfaces. This problem is currently the subject of considerable controversy. Previously, the present author has suggested that depth of orientations of the order of magnitude of 0.1 micron (i.e. $10^{-5}$ cm) may be common, particularly in biological systems (Drost-Hansen, 1971). Other evidence for vicinal structuring of this order of magnitude has come from measurements of energies of activation for ionic conduction in narrow capillaries (see Schufle and Yu, 1968; and Schufle, Drost-Hansen and Huang, 1972; also Drost-Hansen, 1969c). Claims for even greater depth of oriented water layers have come from such notable investigators as Schulman and Teorell (1938); these authors determined the amount of water moving with a monolayer on an aqueous surface in the form of a "rigid layer." By the technique of flowing monolayers, an apparent boundary layer thickness of thirty microns was determined! It would appear that some hydrodynamic effect could have been operating which was not taken into account by Schulman and Teorell. However, Blank and LaMer (1957, 1958) have also claimed the existence of extensive water layers beneath monolayers on an aqueous substrate. It is interesting that highly anomalous surface viscosities for monolayers have been reported frequently. Some of these have shown anomalous temperature dependencies, suggesting relatively abrupt melting of the water structures beneath the monolayer (see Drost-Hansen, 1972).

The present author is skeptical about depths of orientations greatly exceeding a fraction of a micron but it is frequently difficult to find valid objections to the measurements reported. The measurements by Simons and Coster—suggesting that adjacent to bimolecular leaflets (see Section III.B.8) the water might be affected to a distance of about 0.1 to 4 microns—appear quite conclusive, although Schwan (1972, personal communication) has proposed that fluctuations in the excess electrolyte impedance may be the cause of the observed anomalous behavior. However, when all the presently available evidence is considered, it appears that 0.1 microns is a relatively safe estimate for the extent of structural effects, but is necessary to delinate very clearly what is meant with such "long-range ordering."

### 4 *Discrete structures vs. temporal stability*

As stressed earlier, the structure of bulk water continues to escape a reasonable description. Thus, it is not yet known whether a "continuum model" or a "mixture model" is best suited to describe the structural characteristics of liquid water. In view of this, it is difficult to discuss specific structural attributes of water adjacent to an interface. Yet, the known existence of the high pressure ice polymorphs and of clathrates strongly suggest that water molecules possess the ability to pack or rearrange themselves readily into a multiplicity of fairly stable lattices under the proper circumstances (see Jeffrey, 1969; also Szent-Györgyi, 1971). Thus, it is necessary (assuming that the vicinal water layers adjacent to most any solid retain some fluidity and hence some of the attributes of a liquid) to discuss the problem of temporal stability vs. extent of geometrically identifiable attributes in water. For the bulk structure such a discussion is given in the monograph by Eisenberg and Kauzmann (1969); in the case of vicinal water, it appears that a decision must be made as to whether or not the water near an interface is geometrically different from the structures—if any—found in bulk water. In other words, does water near an interface tend to manifest geometrically identifiable attributes, such as hexagonal rings, pentagonal dodecahedra, etc., which do not exist in bulk water? In the alternative, does structuring at the interface mean merely the stabilization of some more or less well-defined, geometrically identifiable entities already "latently" existing in the bulk liquid, but with slightly longer life times near the interface? The lifetimes of the flickering clusters in bulk (of approximately $10^{-11}$ seconds)—assuming a mixture model for water—may, in the vicinity of a solid, be increased by perhaps one or several orders of magnitude. Such an increase in lifetime will undoubtedly serve to convey measureably different attributes to the vicinal water. One important aspect in this connection remains to be discussed (see Section V), namely the energetics as compared to extent of orientation. In order to introduce this topic, it is necessary first to consider the occurrence of thermal anomalies in the properties of water adjacent to interfaces.

## IV THERMAL ANOMALIES IN AQUEOUS PROPERTIES

### A Polymorphism of vicinal water

As indicated in the preceding section, a large number of possible structured forms of water exist. In view of the abundance of such structured entities, it is little wonder that transitions between differently structured entities can readily occur. In fact, it is remarkable that the occurrence of thermal anomalies in the properties of some aqueous systems was not predicted long ago on this basis! It is even more remarkable that, once described, the notion of thermal anomalies† has met with considerable disbelief or utter disinterest. The topic of thermal anomalies was never particularly appreciated. Writing of most authors were ignored. The only notable exception is the monograph by Dorsey (1940), who faithfully quoted the few, more substantial reports of anomalies, and the writings of Magat (1935, 1937). Without a doubt, Magat was the first author of any reputation to lend stature to this field. In the following sections, a number of thermal anomalies are discussed, all related to water near interfaces. Of necessity, the selection of examples is limited; for various reviews of other evidence, the reader is referred to previous articles by the present author (see 1969, 1971 for summaries and references to earlier works).

### B Examples of thermal anomalies in aqueous, interfacial systems

#### *1 Disjoining pressure of water between quartz plates*

Peschel and Adlfinger (see 1971) have, over the past several years, devised an exceedingly precise, conceptually simple, but instrumentally ingenious, device for determining properties of liquids between two highly polished, quartz surfaces. This instrument was used to obtain the viscosity data referred to in Section III.B.7. The instrument has also been used for the original objective of determining disjoining pressures (for a discussion of

---

† Such termal anomalies have, in the past, been referred to as "kinks" (and, by some authors, as "discontinuities").

disjoining pressure, see, for example, Sheludko, 1966); Peschel and Aldfinger, 1968; and Padday, 1970).

The results of a number of measurements at different plate separations are shown in Figure 4. The experimentally observed disjoining pressure is shown here as a function of temperature for three different plate separations. Note the remarkably pronounced maxima and minima of this quantity as a function of temperature. For plate separations of 300 Å and larger, anomalies are clearly seen at the temperatures discussed previously by the present author (namely, around 15, 32, 45 and 61°). (For a very small separation between the plates, 100 Å, other thermal anomalies are indicated, possibly around 8, 12, 23, 35, and 50°C). Note that for all plate separations, the disjoining pressure decreases to zero at about 74°.

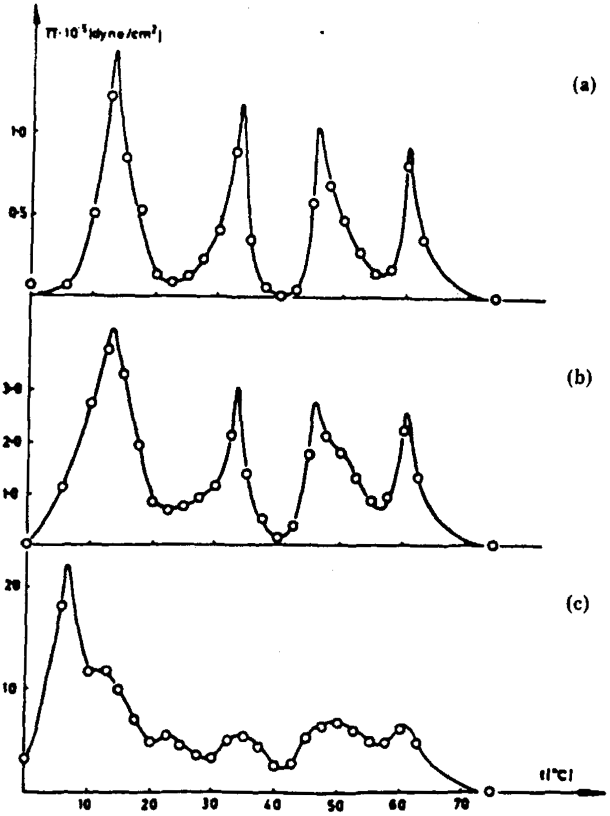

FIGURE 4 Disjoining pressure of water between quartz plates, separated (a) 500 Å; (b) 300 Å; and (c) 100 Å, as a function of temperature. (Peschel and Adlfinger, 1971).

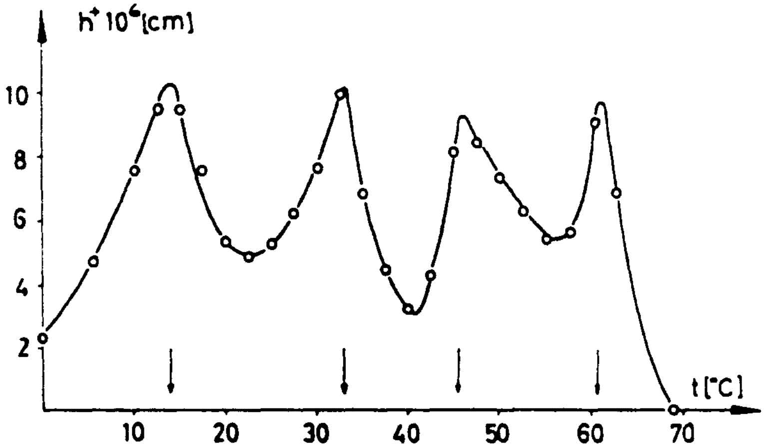

FIGURE 5 Distance at which disjoining pressure is $10^4$ dynes/cm$^2$, as a function of temperature. (Peschel and Adlfinger, 1971.)

Figure 5 shows the temperature dependence of the distance for which the disjoining pressure possesses an arbitrarily selected value of 10,000 dynes/cm$^2$. In this illustration, note that at the temperatures of the thermal anomalies, the distance involved is of the order of 0.1 microns! Together with the disjoining pressures themselves, this information must be of considerable interest in many problems, such as kinetics of flocculation, cell adhesion, etc.

### 2 *Diffusion coefficient for thiourea*

Only rarely have diffusion coefficients been measured at closely spaced temperature intervals. However, recently Dreyer, *et al.* (1968, 1969) have devised a unique and elegant method for rapid determination of diffusion coefficients. Figure 6 shows results obtained for the diffusion coefficient of thiourea in water. The authors call attention to the occurrence of unusual abrupt changes near 13, 28, 43, and 60°, and also note that these temperatures agree (within a few degrees) with those previously suggested by the present author on completely different experimental basis. Dreyer and co-workers emphasize that the anomalous behavior most likely is due to influences of the capillary walls, i.e. the phenomenon is not a bulk effect. This also points to the danger of generalizations based on measurements of diffusion coefficients in biological systems, unless such measurements are performed at closely spaced temperature intervals. The anomalous temperature dependence obviously makes the application of any simple transport theory difficult or impossible (such as an analysis of the data by Arrhenius expression or an Eyring-type rate expression).

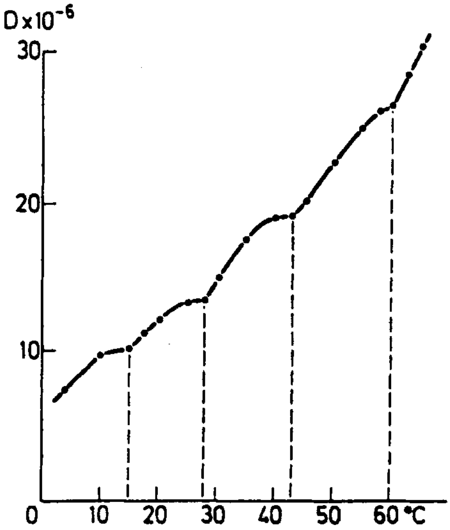

FIGURE 6 Apparent diffusion coefficient of thiourea in water as a function of temperature. (Dreyer *et al.*, 1969.)

### *3 Surface and interfacial tension of water*

Highly precise surface tension data for water were obtained by a capillary rise method as early as 1847. These and many more recent results clearly indicate the occurrence of one (or more) thermal anomalies (see Drost-Hansen, 1965b, 1972b). It appears that many of those measurements of surface tension which involve the three phase system—solid/water/air—may give rise to spurious results showing notable thermal anomalies in the apparent surface tension values. Thus, the present author has demonstrated very dramatic, abrupt changes in the surface entropy of water, calculated from capillary rise data. More recent work, however, by Gittens (1969), by Johansson and Eriksson (1972), and by Cini and co-workers (see, for instance, 1969, 1972) have demonstrated that systems representing essentially *only* an air/water interface do not show any evidence of thermal anomalies. On this basis, the present author has suggested that the anomalous results frequently reported over the past 125 years are likely real but artifacts in the sense of being caused by structural changes of water at the point of three-phase contact (particularly where glass and/or quartz surfaces have been involved). It is notable that the capillary rise data obtained by Myers and Drost-Hansen (see Drost-Hansen, 1965b) gave rise to apparent entropies of surface formation which increased by a factor of two (!) over an interval of temperature of only a few degrees.

### 4 *Ultra-low frequency relaxation of vicinal water*

Using a method first proposed by Thurn (1963), Forslind (1966) demonstrated remarkable temperature dependent anomalies in the damping of vibrating quartz capillaries filled with water. One such set of results is shown in Figure 7. The half-life of the vibrations (in seconds) of a 30 cm long quartz capillary (bent in the shape of a U-tube) is shown as a function of temperature. A notable peak in the half-lives is observed near 32°. Using a somewhat similar approach, Kerr (1970) has verified the anomalous behavior near this temperature (and in some cases, at other temperatures). Kerr further observed that the electrical conductivity of the water in such a vibrating capillary also showed a remarkable minimum in the vicinity of 28 to 32°. The present author (1971, 1972) has proposed the following explanation for the damping effect: below approximately 28°, one type of (extensive) water structuring is present, adjacent to the quartz capillary wall. The mechanical energy of the vibrating capillary is dissipated in viscous processes in the "interior" of the capillary, coupled to the quartz via the structured entities at the walls. Above approximately 32°, another type of (extensively) structured entities are present, again effective in "coupling" between the vibrating quartz capillary and the viscous dissipation processes in the capillary. In the interval between 28 and 32°, enhanced disorder prevails, probably resulting in an increase in smaller, less structured vicinal entities with a concommitant decrease in the mechanical coupling between the

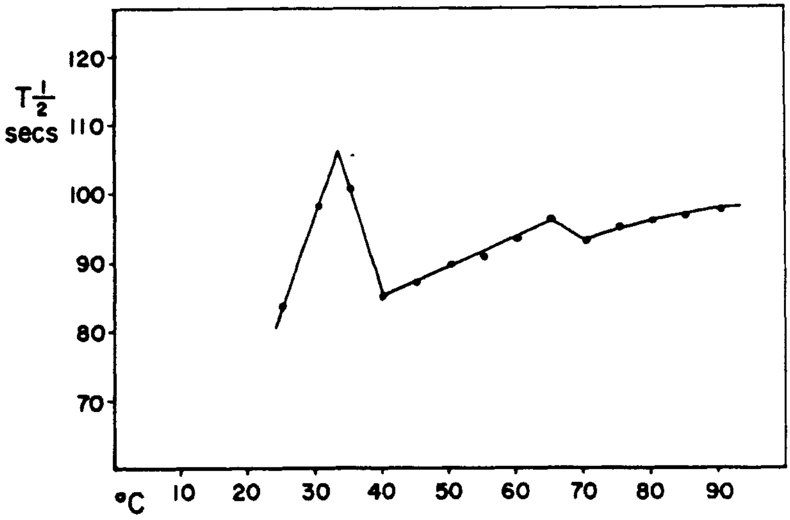

FIGURE 7 "Half-life" of vibrations of oscillating, water-filled, quartz capillary tube. (Data by Forslind, 1966.)

confining quartz walls and the water in the capillary. This explanation is consistent with the increase in surface entropy around 30°, as deduced from the surface tension measurements on water determined from capillary rise in glass (or quartz) capillaries. Furthermore, this is possibly also consistent with the enhanced disjoining pressure over the narrow temperature range. Note that the differential molar excess entropy (as calculated from the disjoining pressure data by Peschel and Adlfinger) changes sign at about 33° (see also Section V.C and E).

### 5 *Dielectric properties of some aqueous systems*

Anomalous dielectric properties of water as a solute in benzene, cyclohexane and carbon tetrachloride have been observed by the present author (1969). These anomalies are manifested in the temperature coefficients for the cell capacitance, changing sharply in the vicinity of some of the temperatures enumerated before. It is unfortunately not yet possible to determine if these anomalies are due to unique interactions between structured entities of water as a solute in these organic solvents, or a (spurious) manifestation of structured water adsorbed on the cell walls of the capcitance cells employed. However, see also Karyakin, *et al.* (1970) who proposed, on the basis of IR studies, that water might exist simultaneously (for instance, in $CCl_4$) as monomeric water and in a "micro-emulsified state." (See also Lawrence, 1969).

For water adsorbed from the vapor phase onto a polymeric surface somewhat similar, relatively abrupt anomalies have been reported by Lubezky, *et al.* (1967). The loss tangent of water adsorbed on chondroitine-4-sulfate shows remarkable peaks at approximately 34 and 61°.

Most recently, Ballario, *et al.* (1971) have observed anomalous variations in the dielectric properties of water near polystyrene particles. Ballario, *et al.* show the loss factor (measured at 10 GHz) as a function of temperature; the data points fall on two distinct, different curve segments (with slightly differing slopes) with a transition region between 28 and (approximately) 33°. On this basis, it is possible to calculate that approximately 7% of the volume of water in the suspension (below 28°C) is somehow structured by the proximity to the surfaces of the spheres, while above approximately 34°, this structured water seems to disappear.

Previously, Johnson, *et al.* (1967) had presented evidence for abrupt anomalies in both spin-spin relaxation times of water adjacent to polyvinyl alcohol particles (0.8 microns in diameter) and in the flocculation rate for polyvinyl toluene particles. This finding is eminently consistent with the idea of structured water at such polymer/water interfaces as described, for instance, by Ballario, *et al.* It should be noted, however, that subsequently the Unilever Research Laboratory workers (Clifford, *et al.*, 1970) have failed to confirm this effect. The authors concluded on the basis of their more recent study that there was no evidence for long range effect of uncharged surfaces on the water structure (except for possibly a depth of one or two layers). The present author finds it difficult to accept this more recent conclusion in view of some of the evidence available from other studies—in part, discussed in this paper. It is particularly unfortunate that Clifford, *et al.* have not shown their results for the spin–lattice relaxation data or spin–spin relaxation data as functions of temperature. A casual inspection of their figures suggest the possibility that the temperature dependence remains anomalous.

Finally, in connection with the problem of dielectric studies on aqueous solutions, attention is called to two papers by Shepherd and Grant (1968a,b). These authors studied the dielectric properties of aqueous solutions of proline and hydroxy-proline over a wide range of frequencies (about 100 to 2,000 MHz) as a function of temperature. It is of interest that at low temperatures (below 20 and below 30°, respectively, for hydroxy–proline and proline), evidence is obtained for "vicinal water" which disappears above the temperatures listed. Furthermore, this (presumably structured) water near the amino acid molecules has a very high temperature coefficient for the relaxation wavelength. (We return to this problem in the section (VII.B.2.c) on thermal anomalies in protein solution—notably collagen).

### 6 *Anomalies in properties of aqueous membrane systems*

In a recent article (Drost-Hansen, 1971) the interactions between membranes and water were discussed in some detail. Only two examples will be mentioned here to illustrate the type of results frequently observed.

Ilani (1965) has introduced a conceptually simple type of hydrophobic membrane. The membrane system consists of a porous cellulose matrix (such as a Millipore filter) saturated with an organic, water-immiscible liquid. In our own laboratories, we have used this method, particularly using Millipore filters saturated with toluene. Figure 8 shows the concentration potential as a function of temperature for such a system, namely, an "Ilani" membrane, separating two potassium chloride solutions, respectively 0.1 and 1.0 molar. An abrupt change in the temperature coefficient is observed at 30°. Similarly, measurements of the resistance of such a membrane have also shown an anomalous temperature dependency. This is indicated in Figure 9. The two curves correspond, respectively, to gradual heating and gradual cooling of the system. A small amount of hysteresis is observed, possibly reflecting—among other factors—changes in mutual solubility, toluene-water. For other highly unusual temperature dependencies of membrane properties, see, for instance, the studies by Nelson and Blei (1966).

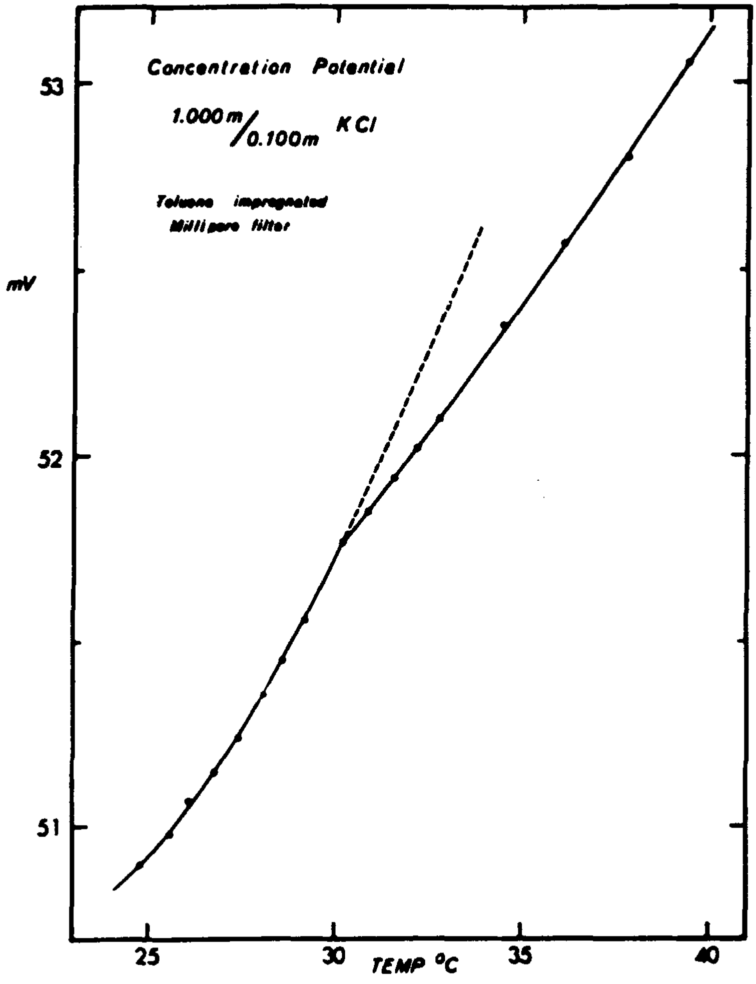

FIGURE 8 Concentration potential across toluene impregnated "Ilani" membrane.

## C Paradoxical effect

### *1 Non-specific effects of interfaces*

Thermal anomalies have been observed in the properties of vicinal water adjance to quartz, glass, lead iodide, cellulose, polymers (such as polyvinyl alcohol, polyvinyl toluene, chondroitin-4-sulfate), pure hydrocarbons, fatty acids, clays, etc. In other words, thermal anomalies have been observed for water adjacent to most any type of surface, regardless of the detailed chemical nature of the solid (or liquid). In particular, it is remarkable that

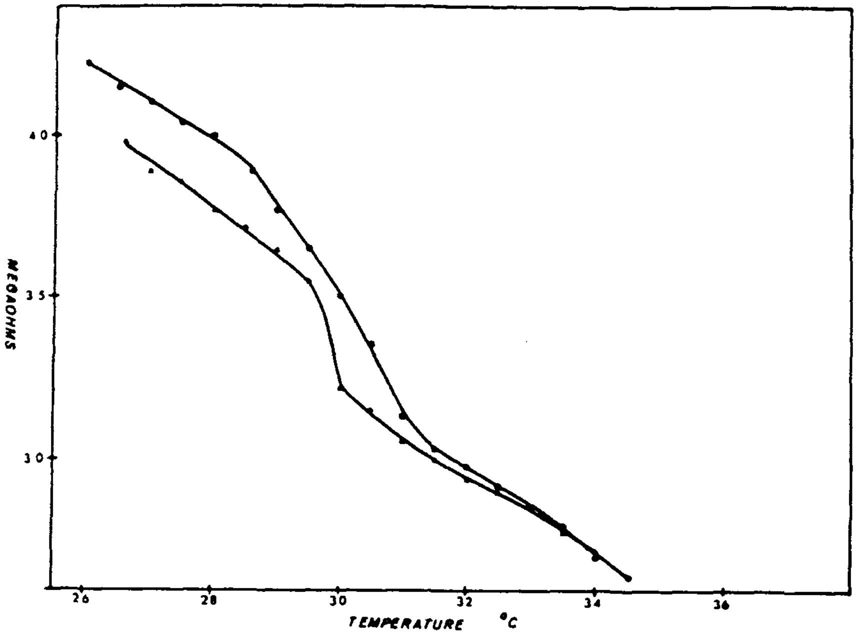

FIGURE 9 Resistance of "Ilani" membrane as a function of temperature.

the thermal anomalies appear independent of whether the solid is hydrophobic or hydrophilic! As this is indeed rather unexpected, the present author has referred to this phenomenon as the "paradoxical effect."

## 2 *Possible substrate-independent stabilization of water structures*

No obvious, immediate explanation comes to mind for the paradoxical effect. However, it has been proposed (Drost-Hansen, 1971) that structured entities of water near any interface may be come stabilized by an "energy delocalization effect." In this mechanism it is assumed that one (or more) of the possible structured types of water discussed previously may become stabilized merely by promixity to an interface. In bulk water (or solution) thermal fluctuations will incessantly disrupt the (possible) structured entities, leading to constant breakdown (and short-lived restructuring). However, in the vicinity of a solid, it appears that a high-energy fluctuation—which in the bulk would have disrupted a "critical" hydrogen bond in a cooperatively hydrogen bonded structured species—may be "shunted away" to the solid. In other words, it is proposed that high-energy fluctuations in the vicinal water may be redistributed over different (mostly vibrational) modes of the adjacent substrate. Obviously, no net gain in vibrational energy can be experienced by the substrate and it is therefore necessary to assume that the energy thus imparted to the adjacent material is subsequently re-dissipated over the neighboring liquid structures, but dissipated over a larger number of hydrogen bonds.

*a Microscopic reversibility* Professor Friedman (1972) has questioned whether or not this picture is consistent with microscopic reversibility. It seems to the present author that the principle of microscopic reversibility need not be violated. Thus, in principle, the system somewhat resembles an equilibrium (say, at the triple point) between a solid and its melt. In the latter system, both momentum and material exchange will occur at the interface. In the case discussed in the paradoxial effect, only a momentum effect is invoked (since, in this case, the solid and the "melt" are chemically different species).

*b Hydrophobic bonding* The "energy delocalization effect" resembles, to some extent, the basic idea invoked in hydrophobic bonding. In the latter case, stabilization of water results from the added presence of a fifth neighbor to the generally four-coordinated water molecule. The presence of this additional neighbor lowers (via the van der Waal interactions) the energy levels of the water molecules. In the case of the paradoxical effect, the addition of a "half space" of (ordered) molecules (atoms) lends stabilization to the vicinal layers of water molecules via a redistribution of kinetic energy.

*c Non-equilibrium aspects* In connection with the problems inherent in the energy delocalization effect, attention is called to the study by Fröhlich (1968), (see Section VIII.B) who has specifically suggested the possibility that in cases far from equilibrium, energy may not be thermalized, but rather stored in a single vibrational mode leading to long-range phase correlation. It is not clear, however, at the moment how this mechanism might apply in a thermodynamic *equilibrium* case as discussed in the present section. On the other hand, if the energy of a system (under special circumstances) need not be thermalized in the traditional sense, it becomes possible, perhaps, to rationalize how two individual, discrete states may co-exist (differing in thermal energy by less than approximately $\frac{1}{20}$ $RT$.)

It is difficult to present a convincing argument for the energy delocalization effect. The experimentally observable macroscopic manifestation to be explained is the stabilization of presumably rather extensively structured entities, regardless of specific chemical interactions between the vicinal water and the solid. The explanation in terms of energy delocalization begins with a qualitative, microscopic picture of molecular dynamics—at that, ill formulated at his point. From molecular dynamics, it is difficult indeed to deduce macroscopic transport properties and it is well nigh impossible at the present to obtain equilibrium thermodynamic quantities for such complex systems. (See, for instance, Wightman, 1971). However, for lack of any other likely mechanism the "delocalization effect" is proposed here as a temporary, working hypothesis.

*d Absence of electrolyte effects* Without presenting the detailed experimental evidence, we state merely a general observation regarding the thermal anomalies. The temperatures at which the thermal anomalies occur appear essentially unaffected by the presence of both electrolyte and nonelectrolytes in solution. In other words, the temperatures of the thermal anomalies are unaffected by the presence of salts (at least up to concentrations of about 1 molar) and unaffected by the presence of most non-electrolytes, often up to considerably higher concentrations. Essentially, only high concentration of hydrogen ions significantly affects the temperatures of the thermal anomalies. In this connection, compare the results by Safford (1966) on the persistence of lines observed with pure bulk water in the neutron inelastic scattering spectra, obtained on concentrated electrolyte solutions.

*e Irrelevance of electric double layers for vicinal water structuring* It is important to recognize that in non-living systems, in true equilibrium, the properties (and hence the structure) of water near an interface are often notably different from those of ordinary bulk water. Near an interface, the water appears extensively ordered, possibly to a depth of, say, a tenth of a micron—in other words, over distances which are indeed very large compared to a molecular diameter. The thermal anomalies are also present in vicinal aqueous solutions of electrolytes, but the observed, anomalous properties are *not* due to electrical double layers. At room temperature, the Debye characteristic thickness ($1/\kappa$) is of the order of 0.1 microns for a $10^{-5}$ molar solution. This is thus of the same order of magnitude as the ordered water structure discussed here. However, as mentioned above, the thermal anomalies remain unaffected in the presence of electrolytes up to about one molar concentration, for which $1/\kappa$ is completely negligible (for a $10^{-3}$ molar solution of a 1–1 electrolyte in water at 25°, $1/\kappa$ has already decreased to only 10 Å). In other words, since the vicinal water structure retains its characteristic long-range ordering in the presence of electrolytes, the anomalous properties of the water near the interface is not a manifestation of a double layer phenomenon!

### 3 *Other evidence for non-specific solid/water structure effects*

Other evidence is beginning to accumulate suggesting a "non-specific" type of stabilization of water adjacent to various interfaces. Thus, Dehl (1968) studied the properties of water and heavy water adsorbed on parallel fiber bundles of crystalline rayon. The main result of this study was the conclusion that water molecules may be influenced by proximity to the rayon, so as to allow rapid reorientations about all possible axes, but "maintain a small resultant projection, along the fiber axis." Dehl also quotes Odajima (1959), Zimmerman and Lassater (1958), Woessner and Zimmerman (1963) and Graham, Walker and West (1964) all of whom had observed anisotropic rotation of water molecules near various interfaces. These studies encompassed materials as different as cellulose, silica gels, and silicates. On this basis, Dehl concluded "the observation of anisotropically rotating water molecule in such a diverse array of materials as proteins, cellulose, silica gel, and zeolite surely suggests that a common explanation cannot depend much on the details of molecular geometry of the crystalline lattices. Instead, the present work suggests that the water orientation phenomenon may be simply explained by rapid, but not quite spherically symmetrical, rotation of water molecules on an asymmetric crystalline lattice." The present author does not necessarily subscribe to the proposed explanation in terms of "rapid, but not quite spherical symmetrical rotation." However, it is of interest to note that Dehl concluded that some general, non-matrix determined effect appeared to prevail at the water/solid interfaces.

More recently, Woessner arrived essentially at the same result, concluding that "these results appear to show that the presence of a surface is generally more important than its nature in determining many structural and dynamic aspects of water at or near the interface" (Woessner, 1971, personal communication).

### 4 *Anti-crystalline structures and liquid crystals*

Ubbelohde (1965, 1971) has introduced the concept of "anti-crystalline" liquids. In his report to the XIV Solvay Conference, Ubbelohde observes "if several types of molecular assembly are, in fact, feasible in a liquid, no single parent crystal structure any longer provides a complete guide to the liquid structure. Liquids with this higher degree of molecular complexity which cannot usefully be referred to in any single crystal lattice, are conveniently described as anti-crystalline." It is proposed here that the types of water structures stabilized by proximity to almost any solid may correspond to the anti-crystalline entities envisioned by Ubbelohde. Before proceeding, note that in the present case, however, the comparison is not between a crystalline solid in contact with its melt, but rather a different type of solid adjacent to (possibly several) different anti-crystalline structured elements of water. (The possible existence of types of structured, "slushy ice" at the ice/water interface [during growth of an ice crystal] has been discussed by the present author, 1965). Ubbelohde notes that on freezing, only one of the anti-crystalline "isomers" may be selected for building into the appropriate crystal lattice. It seems possible that *none* of the anti-crystalline structures are compatible with the solid form of water (i.e., ice-Ih). Thus, a prevalence of differently structured, anti-crystalline entities may be consistent with a mechanism of heterogeneous nucleation not involving nuclei of Ice-Ih formed by epitaxi in heterogeneous sytems. In the terminology of anti-crystalline vicinal water structures, the temperatures corresponding to the thermal anomalies are the temperatures of abrupt transitions from one predominant structured species to another type of anti-crystalline entity.

To appreciate the possible existence of a multitude of somewhat differently structured units of water near interfaces, a brief review is presented of solids exhibiting several different polymorphic forms. This review is borrowed rather directly from the Monograph by Ubbelohde (1965).

1-2-4 trimethyl benzene exhibits two different, distinct melting points, namely 229.3°K (solid I) and 224.2°K (solid II). The two solids apparently melt to give similar and possibly identical type melts. According to Ubbelohde, this anomaly has not been resolved; (the structural differences in the polymorphs have not been elucidated; see Putnam and Kilpatrick, 1957).

Both tetraethyl lead and tetraethyl tin appear to possess a large number of polymorphs. The melting points all fall within a narrow range of temperatures. Thus, for the tin compounds, no less than ten polymorphs have been claimed, melting in the range from 137.4 to 147.1°K. Six polymorphs have been claimed for tetraethyl lead (melting points from 135.6 to 141.5°K). No such polymorphism appears to exist with tetramethyl lead or tetramethyl tin, nor with methyl or ethyl derivatives of carbon, silicon or germanium. The polymorphism in the tetraethyl tin and lead compounds may be due to rotational isomerism.

Ubbelohde mentions that some soaps, such as sodium palmitate, exhibit at least five successive phase changes below the appearance of the isotropic melt ($T_f$) less than 292°C (Wold and Wold, 1939). In connection with the mentioning by Ubbelohde of sodium palmitate, see also the more detailed discussion of lipids and water/lipid interactions discussed in Section VII.B.2.a.

All of the above observations are of interest in connection with the claim that at least four thermal anomalies appear to exist in water. Admittedly, in the problem of water near interfaces, we are dealing with what is presumably a liquid system, as opposed to the solid polymorphs discussed above. However, as mentioned in Section III.B, the vicinal water may possess attributes which make it more solid-like than liquid—possibly being anti-crystalline. An explanation in terms of solid attributes should undoubtedly not be carried too far, if for instance, the specific heat of vicinal water is still close to one. Confer, however, the notable enhancement in heat-conductivity discussed in Section III.B.6.

The thermal anomalies may be considered as critical phenomena. In one such approach, one might consider the water immediately adjacent to the solid surface to represent a liquid phase which, at any given temperature and pressure, is in "equilibrium" with an anti-crystalline "fluid" vicinal to it. Within each temperature interval one structure or another may predominate. Thus, as temperature is increased, a critical transition occurs in going from one type of stable (anti-crystalline) configuration of the vicinal water to another (anti-crstyalline) arrangement. The higher temperature form will probably possess greater symmetry (and likely smaller size). Naturally, these speculations leave completely unspecified the molecular nature of the anti-crystalline, structured elements and particularly (and unfortunately) the nature of the "transition composition." It would seem that in the transition region, for instance, between 29 and 32°, an additional large entropy contribution should be expected, due to the "mixing" of the two different possible structures with a third, far more disordered smaller entity. One such obvious link between the structured entities would naturally be monomeric water molecules. Another possibility is to consider the structured elements as solutes (in a phase of bulk structure), positively adsorbed at the interface. Again, as temperature is increased, there is a transition in the "adsorbed," vicinal structured elements to another type, again presumably of higher symmetry, but smaller size.

As mentioned earlier, Ubbelohde has noted that there were several types of molecular assembly possible in the liquid phase, the structure of a single parent crystal can no longer completely determine the liquid structure. In other words, liquids with enhanced molecular complexity (not possessing merely a single crystal lattice) may conveniently be described as "anti-crystalline." Examples already cited above include, for instance, chains of flexible molecules, which possess a large number of configurational isomers. In this connection, note also the observation of the shrinkage in volume on melting of certain metals such as galium, antimony and bismuth. Ubbelohde suggests that this points to a pronounced anticrystalline structure for the melts of these metals. Note that shrinkage in volume is, of course, encountered in ordinary water. It would be of interest to study carefully the density of vicinal water from this point of view.

## D Structural aspects of vicinal water and the implications of thermal anomalies

### *1 Historical note regarding vicinal water*

A brief, historical note is appropriate at this point. Thermal anomalies in the properties of both water and aqueous solutions have been reported in the literature for about half a century. The topic was never highly esteemed and some papers on the topic were less than dispassionate and objective. Interestingly enough, the various claims for the existence of thermal anomalies led to significant, careful and precise measurements—most of which failed to reveal any evidence for the occurrence of anomalies (in bulk systems). Only over the last few years has the topic begun to attract some attention and attain some degree of respectability. The change in attitude seems primarily to have followed the disclosure that thermal anomalies likely do *not* occur in bulk water (nor in most bulk, aqueous electrolyte solutions), but are instead strictly a manifestation of an interfacial phenomenon.

### *2 Multiplicity of vicinal water structures*

At least four thermal anomalies in interfacial properties have been identified between the freezing and boiling point of water. Hence, it must be assumed that at least five different structured species exist. Occasional anomalies have been reported at other temperatures. Thus, for very small separations between two confining quartz plates, anomalies in the properties of the water appear, for instance, to occur also at 23° (see Section IV, B.1). For aqueous lipid systems, anomalies are repeatedly reported at around 37 (to 40°C.) Notice also that the disjoining pressure disappears for temperatures above 74°. Finally, a number of claims have been made for anomalies in biological systems at 23° and 37°. The author reserves his opinion as to the cause of the anomalies. However, there is little doubt that a large number of differently structured, stable entities of water structure may occur near interfaces. As mentioned in Section IV.A, perhaps this, should not at all be surprising in view of the multiplicity of polymorphic forms of ice and the large number of known clathrate hydrates.

Related to the general problem of water structures near interfaces, attention is called to the recent study by Franks, *et al.* (1969) on neutron inelastic scattering by aqueous solutions of tertiary butyl alcohol. There appears definitely to be a structural effect associated with the presence of this solute in water. While completely miscible with water, the tertiary butyl alcohol does represent a strongly hydrophobic environment to the water. It is interesting that the lines in the NIS spectra are sharpened, but that no large changes appear to take place in the position of these lines. In other words, it appears as if the water structure becomes more firmly established than the original water structure.† Perhaps this simply represents the slowing down of local motions. For this reason, Franks, *et al.* have referred to these structures as "glassbergs." This phrase seems less prone to misinterpretations than the term "icebergs" coined by Professor Frank. The notion of glassbergs—the stabilization of "glassy attributes" of water adjacent to solute—is of particular interest in connection with the proposed energy delocalization effect for vicinal water structure stabilization discussed in the present paper. Franks, *et al.* stress that the water in these solutions definitely does not appear "icelike"!

### 3 *Higher-order phase transitions and extrema in bulk properties of water*

The occurrence of a higher-order phase transition in a homogeneous bulk liquid is not expected. (Although highly unusual properties appear to have been observed for the liquid phase of xenon hexafluoride; see Anonymous Note, Nature, 1970). The point of the present paper is that the water near a solid interface does not possess the attributes of a bulk liquid phase and hence, the occurrence of thermal anomalies does not violate any generally accepted rule for bulk behavior. The existence of at least four thermal anomalies is, furthermore, perhaps no more surprising than the large number of different extrema observed in the properties of bulk water (for instance, maximum density at 4°C; minimum in specific heat at 35°; minimum in compressibility at 40 to 45° or maximum in ultrasonic velocity at around 75°).

In fact, the existence of such a large number of extrema in the properties of bulk water is possibly a good indication that the structure of water is best described in terms of a mixture model. As discussed briefly by the present author, the very gradual and shallow minima and maxima in various properties of bulk water may be manifestations of gradual changes in proportions of differently structured types of (bulk) species. This would suggest the simultaneous existence, at any temperature, of several different types of structures. If these speculations should prove correct, it becomes easier to understand how some of these specific structures may selectively gain stability by proximity to a solid interface. Note finally, that regardless of whether or not the structure of bulk water is best described in terms of a continuum or mixture model, the evidence for thermal anomalies in the properties of vicinal water is quite convincing and from this one must conclude that at least vicinal water likely possesses distinctly structured entities.

---

† A true "structure maker" in the rigorous sense of the phrase.

In Section IV.A, the idea of polymorphism in vicinal water was introduced. The thermal anomalies which have been reviewed in the preceding section may therefore be discussed as manifestations of higher-order phase transitions. In other words, the changes in properties at the temperature regions of transition represent the cooperative phase changes from one structured type of interfacial water to another type. Higher-order phase transitions are obviously not expected in a homogeneous, simple liquid. On the other hand, the evidence presented in Section III.B suggest that the water near many interfaces is far from "normal" and, in fact, may likely possess attributes of a state somewhere between those of a liquid and those of a solid. As discussed in Section IV.C.4, such a state might be the anti-crystalline state discussed by Ubbelohde (1965) or possibly resemble liquid crystals. As far as a statistical mechanical treatment, it is obvious that the difficulties will be enormous. First it must be recognized that no second order phase transition can occur between liquids and crystalline solids (Landau, 1937, see Landau and Lifshitz, 1958) due to the presence of a third order term in the expansion of the thermodynamic potential. Note, however, that we are undoubtedly not dealing with a simple, homogeneous, isotropic system for which the particular physical model is applicable on which the statement by Landau was made. Hence, there appears no a priori reasons to exclude the possible existence of phase transitions in vicinal, structured water.

### 4 *Thermal anomalies viewed as a "melting process"*

The notion of "melting" of water structures near interfaces appears to first have been mentioned by Franks and Ives (1960). These authors determined the interfacial tension between hexane and water and obtained an inflection point for this quantity in the vicinity of 33–34°C. The present author (1965) has also proposed essentially the same description and most recently, Peschel and Adlfinger (1971) have likewise discussed their anomalies in disjoining pressure in terms of a melting process. These authors (as well as the present author) envision several different, distinct "melting points" of various types of vicinally stabilized water structures. A true "melting," of course, would be associated with a latent heat of transition. Such is generally not observed with vicinal water; see, however, the discussion in Section V.D.

### 5 *Various elements of discreteness*

*a Cluster entropies and bulk clathrates* In the preceeding paragraphs (and in earlier papers by the present author), it has been stressed that the abundance of high pressure ice polymorphs and various types of clathrate hydrates offer an extensive array of possible structured entities which might be stabilized by proximity to an interface. It would not seem unreasonable that other structures—not yet identified crystallographically—might also represent stable configurations. The existence of at least four thermal anomalies forces upon us the need for (at least) five differently structured types of "aggregates" to exist. While readily acceptable within the framework of high pressure polymorphs and clathrates, such degree of discreteness is not readily attained in a traditional cluster model, nor in models treating the vicinal water as structures with lattice defects. Obviously, the existence of five differently structured and "crystallographically" different entities is incompatible with a continuum model.

Recent computer calculations have added a new possibility for types of discreteness of vicinally stabilized structures. Burton (1970) has calculated the entropies of small clusters of atoms, based on a simple model. Specifically, Burton calculated the entropies of "approximately spherical" clusters (with face centered cubic structures) by consecutive adding of shells of neighboring atoms around a single central atom. The calculations were performed for clusters containing 13, 19, 43, 55, 79, and 87 atoms. The result is shown in Figure 10 in which the entropy is shown as a function of cluster sizes.

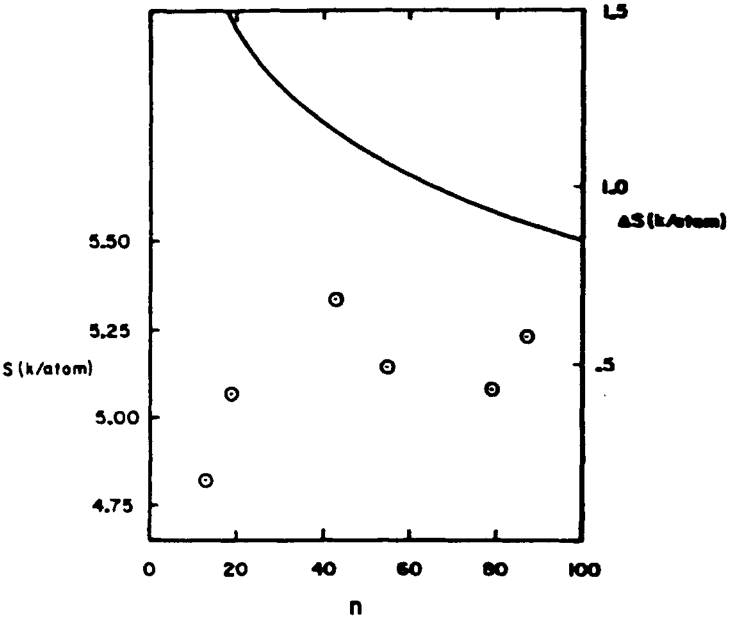

FIGURE 10 Entropy per atom of argon in small, discrete fcc clusters. Circles: exact calculated results. Solid curve: Predicted values for classical drop model. Left hand ordinate: Entropy. S; right-hand axis: difference (ΔS) between cluster entropy per atom and that of bulk phase. (Burton. 1970.)

Obviously, the abscissa in this case is distinctly a non-continuous variable—because of computational limitations. However, the suggestion is that these calculations lead to *non-smoothly* varying entropies. More recently, Abraham and Dave (1971a,b) have extended the calculations. In the first of these two articles, an Einstein model was employed for calculating the "Einstein entropy per atom of the cluster" compared to the value for an infinite Einstein crystal. The difference is referred to as the surface entropy. The approach is based on the idea that for nearest neighbor interactions, the Einstein potential is proportional to the number of nearest neighbors with a frequency dependence to the second power proportional to the gradient of the potential. This gives rise to a scaling of Einstein frequencies as the square root of the number of nearest neighbors; this approach was first invoked by Dickey and Paskin (1970). The results of the calculations by Abraham and Dave are shown in Figure 11. Here the ordinate is the excess entropy per atom (for an argon gas at 93°K). The abscissa is the cluster size. For our purpose, the most important attribute is the "jagged" dependence of the entropy on size. This is the result of the types of cluster packing. The drop in excess entropy for very small clusters is due to the fact that the cluster has 3N-6 modes of motion instead of nearly 3N modes.

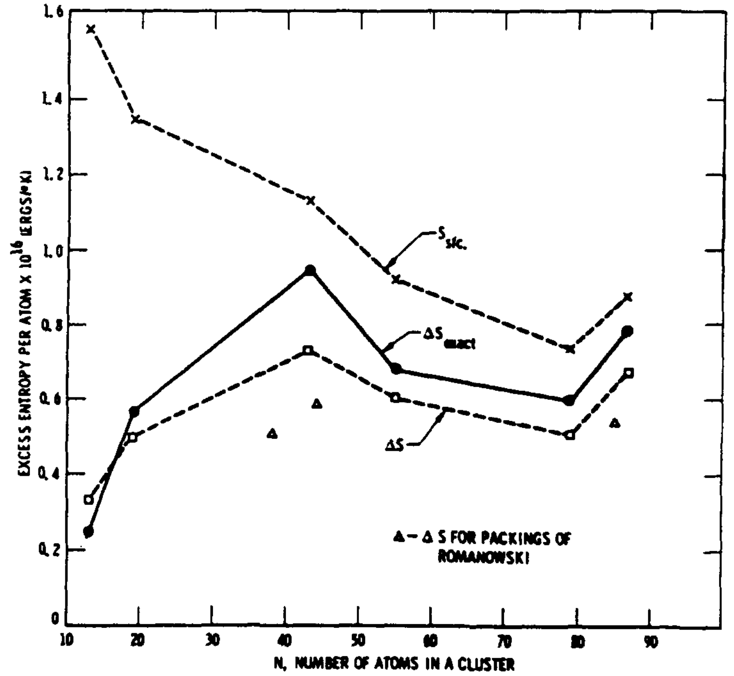

FIGURE 11 Calculated excess entropy per atom for argon gas (at 93°K) as a function of cluster size. (Abraham and Dave, 1971a.)

If the "jagged" distribution of surface entropies (and therefore total entropies of each cluster) is indeed real, the result may be alternate stabilities due to relative minima in the free energy assuming that the enthalpy varies in a smooth function with the (strictly speaking non-continuum variable) cluster size. To make the comparison between gaseous clusters of argon atoms and vicinal water requires a considerable "leap of faith." In view of the prodigious problem facing us (explaining the existence of no less than five different types of vicinal water structures) an existential approach may be justified. Hence, we must interpret the surface entropy as the interfacial entropy between structured (or non-structured) water clusters and a surrounding continuum aqueous structure, even if present only to a very limited extent. The non-structured element may represent a disordered zone in the three layer model previously advocated by the present author. On the other hand, it may indeed be unnecessary to invoke even this state (somewhat similar, incidentally, to the State-III invoked by Frank and Quist, 1961) and merely take the interfacial entropies as those resulting from non-bonding between the different neighboring individual clusters in the vicinal water. It is unfortunately difficult to estimate the degree to which the conclusions, based on the scaling factor employed by Abraham and Dave, is applicable to a structure which is most definitely not a face centered cubic structure. Thus, instead of a "perfect" twelve nearest neighbor configuration, any sort of water lattice is more likely to have a number of nearest neighbors equal to four. On the other hand, in the opinion of the present author, the approach via surface entropies and energies of "micro-crystallites" may offer a more promising approach than the attempts to extend the random packing of sphere model originally introduced by Bernal (1960).

It is possible that the description just presented is merely a different way of expressing the same ideas as represented in the structured entities of clathrate hydrates and/or high pressure polymorphs. It is of interest that Hildebrand (1969) has argued eloquently against the existence of any type of "icebergs" in water, based on a comparison of diffusion coefficients in water and carbon tetrachloride. While the example discussed by Hildebrand appears very convincing (see, however, Drost-Hansen, 1971), it seems incapable of explaining other diffusion data, for instance those reported by Boerboom and Kleyn (1969). The results obtained by these authors for the diffusion coefficients for the noble gases in water are shown in Figure 12. It is obvious that, at room temperature (25°—the temperature for which the measurements by Hildebrand referred) the diffusion constant for argon, krypton and xenon appears practically identical. However, the smaller gases most definitely diffuse relatively much faster than the larger gases. A simple interpretation of these results would be that (at all three temperatures studied), the three heavier inert gases (argon, krypton and xenon) form clathrate hydrates where the aqueous structure is, in each case, probably identical, while the lighter

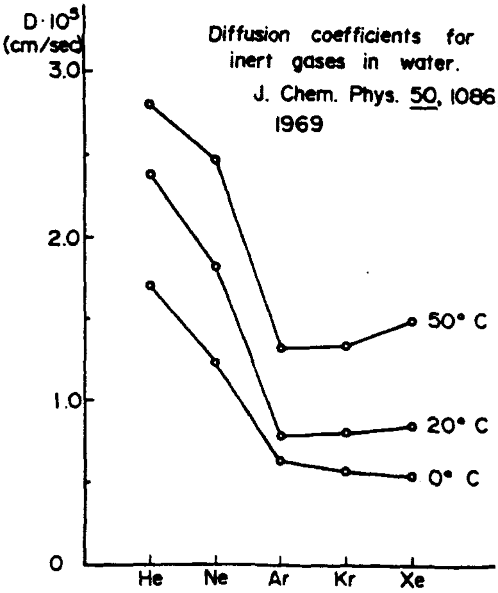

FIGURE 12 Diffusion coefficient for inert gases in water. Abscissa: ordinal arrangement of gases by increasing atomic weight. Temperatures (from bottom curve up): 0, 25, and 50 . (Data by Boerboom and Kleyn, 1969.)

gases (helium and neon) are unable to form such hydrates (or do so to a vastly lesser extent) and hence, diffuse primarily as individual molecular species. Another interesting difference is shown in Figure 13 which displays the apparent energy of activation for diffusion as a function of the nature of the inert gas. It is interesting to note that at 12.5° (average temperature), the apparent energy of activation for viscous flow of water is 4.5 kilocarlories. The apparent energy of activation for diffusion of the noble gases all fall in the range of 2.0 ± 0.6 kcal/gm mole—only one half of the value for the viscous flow of water. Contrarily, at 37.5° (average) the apparent energy of activation for diffusion increases, roughly speaking, linearly with the size of the inert gas, varying from 1.12 kcal/gm mole for helium to approximately 4.55 kcal/gm mole for xenon.

*b Vicinal clathrate structures* The possibility that clathrate-like structures near interfaces may become enhanced remains uncertain. For water adjacent to non-polar solids, and perhaps particularly for water adjacent to macromolecules with non-polar side chains, it seems reasonable to assume that clathrate-like cages might be selectively stabilized by proximity to the interface. The proposals by Pauling and by Miller of anesthetic action in terms of induced stabilization of clathrate cages at neutral surfaces seem

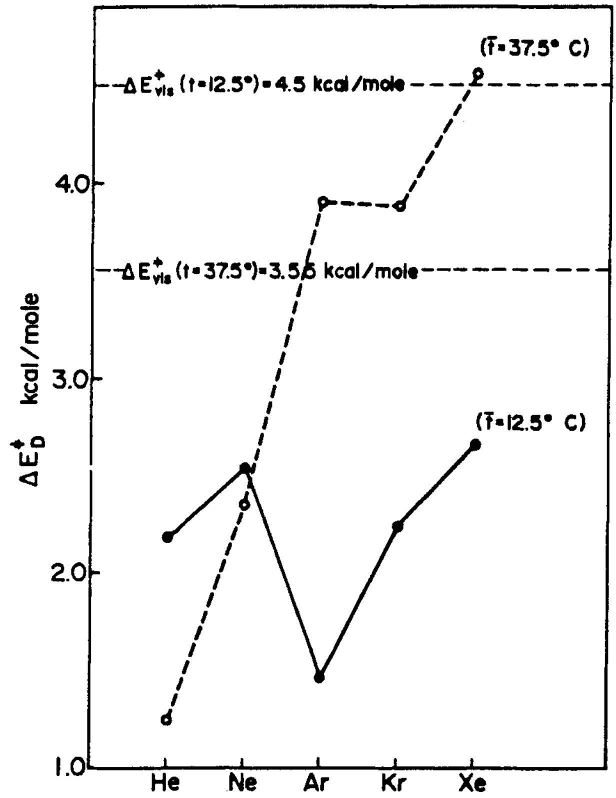

FIGURE 13 Calculated apparent energies of activation for diffusion of inert gases in water. "Average values" at 12.5 and 37.5 for comparison purposes, values of the apparent energy of activation for viscous flow of water are indicated. (Data by Boerboom and Kleyn, 1969.)

ingenious. Presently, however, it does not appear that this suggestion is gaining wide support On the contrary, the older, "competitive theory" of Meyer-Overton seems to be holding its ground (relating anesthetic action primarily to lipid solubility). Somewhat similarly, little evidence is forthcoming to indicate a clathrate-hydrate-like structure for bulk water as envisioned by Pauling, for which a statistical-thermodynamic description was attempted by Frank and Quist (1961). On the other hand, Glew (1962) continues to advocate this model and Arnett, *et al.* (1970) have eloquently summed up the general state of uncertainty with the following remarks: "although we maintain a skeptical attitude towards the existence of clathrates in solution (references 6, 29), we note that the phenomena portrayed in Figure 2 could be explained completely if there were a competition between benzene and the tetrabutyl ammonium bromide for structuring of water.

From pure water to a composition in the neighborhood of the clathrate, structure-making by benzene would become increasingly difficult (endothermic). After the clathrate stoicheometry of the tetra-lakyl ammonium salt had been satisfied, structure-making of water around the benzene molecule could then proceed in the direction of decreasing entropy and exothermic enthalpy. We are uncomfortable with this facile interpretation, but feel compelled to draw attention to it since it fits the facts."

*c Water at the air/water interface* In a recent paper, the present author (1972) has addressed himself to the question of structure in the water beneath a partial or complete monolayer at the air/water interface. The reader is referred to this paper for the details of the ideas and available evidence; we summarize here only the main finding. In the presence of even a rather extended monolayer on an air/water interface, the structure of the water at the surface appears significantly different from that of either the bulk structure or the structure at the (completely "clean") air/water interface. It appears as if the structure of the water beneath the partial (or complete) monolayer strongly resembles the structure of water near a solid interface, discussed in the preceeding sections. As an extreme example, consider the measurements made by Schulman and Teorell (1938); these authors deduced the existence of an effect on the water layers beneath a moving monolayer of oleic acid to a depth of 30 microns! The present author reserves his judgement in this case; however, as pointed out previously, it is interesting that Blank and LaMer (1957, 1958) have confirmed that extensive layers of water are indeed influenced by a monolayer at the air/water interface. Furthermore, studies by Hickman and White (1921) have suggested that even near the boiling point the presence of one insoluble monolayer forming molecule may influence ten (or hundreds) of water molecules at the interface—measurably raising the possible degree of superheating of the water.

*d Surface disruption effects* Chapman has studied the size distribution of micro-droplets formed by bubbling air through water. The results were reported in considerable detail in Loeb's monograph (1958) on "Static Electrification." An example of the results obtained by Chapman is shown in Figure 14. In this illustration, the frequency of occurrence of various sized "clusters" is reported in terms of a vapor phase conductivity (due to charged species of the variously sized micro droplets) as a function of mobility—inversely related to the size of these "micro droplets." From the observed mobilities, Chapman was able to obtain estimates for the sizes of the aggregates. In addition to simple, singly charged water molecules, dimers and trimers, discrete structured entities were detected by Chapman, including aggregates of water consisting of 125, 215, 322, 950, 1300 (etc.) molecules

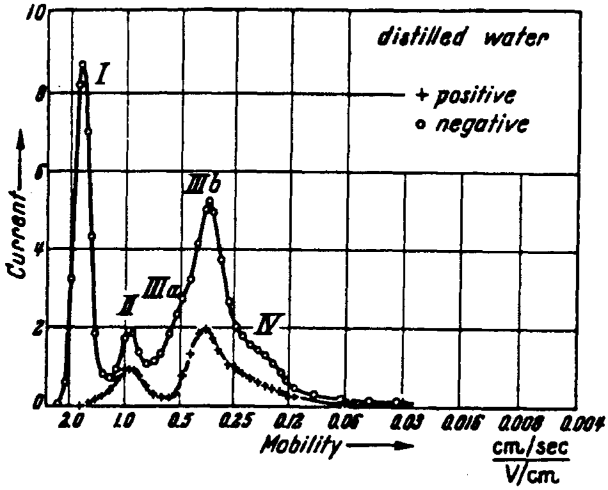

FIGURE 14 Relative concentration of charged species, expressed in relative units across a vertical column of vapor, as a function of mobility (inversely related to "cluster size"). (Data by Chapman; Loeb, 1958.)

(corresponding in radii of 2.5 to 5.5 molecular diameters). A number of larger clusters were reported as well. A number of these discrete peaks are readily observed in Figure 14. It is also seen that the distribution of sizes is somewhat dependent on the degree of local disturbance at the interface. This would suggest that the "relaxation time" of a newly formed air/water interface might be considerably longer than what would be expected from the dielectric relaxation time of $10^{-11}$ seconds (at room temperature). For a brief discussion of the possible relation of this observation to the idea of structured water elements (of considerable size) in bulk water and at the air/water interface, see the article by the present author (1972).

## 6. *Note on structure of bulk water*

We return briefly to the question of the structure of bulk water. The main problem is whether a continuum model or a mixture model offers the best description of the structure of water. Given the existence of thermal anomalies in vicinal water—implying the existence of differently structured entities of water, stabilized by mere proximity to most any type of solid surface—it seems likely that this information should permit some inferences as to the structure of the bulk. The obvious possibilities are: that the vicinal water forms from "almost stable" aggregates, pre-formed in the bulk liquid, or, on the contrary, that the structures are induced, ab initio, from a "random distribution" of water molecules as envisioned in the continuum model.

TABLE III

Thermodynamics of ice–ice transitions†

| Transition | | $T$ | $P$ | $\Delta V$ | $\Delta S$ | $\Delta H$ | $\Delta E$ | $P\Delta V$ |
|---|---|---|---|---|---|---|---|---|
| From | To | (°C) | (kbar) | (cm$^3$ mol$^{-1}$) | (e.u.) | (cal mol$^{-1}$) | (cal mol$^{-1}$) | (cal mol$^{-1}$) |
| I | II | −35 | 2.13 | −3.92 | −0.76 | −180 | 19 | −199 |
| I | III | −22 | 2.08 | −3.27 | 0.4 | 94 | 256 | −162 |
| | | −35 | 2.13 | −3.53 | 0.16 | 40 | 219 | −179 |
| | | (−60)‡ | (2.08)‡ | (−3.70)‡ | (−0.46)‡ | (−99)‡ | (83)‡ | (−182)‡ |
| II | III | −24 | 3.44 | 0.26 | 1.22 | 304 | 283 | 21 |
| | | −35 | 2.13 | 0.39 | 0.92 | 220 | 200 | 20 |
| II | V | −24 | 3.44 | −0.72 | 1.16 | 288 | 347 | −59 |
| III | V | −17 | 3.46 | −0.98 | −0.07 | −17 | 64 | −81 |
| | | −24 | 3.44 | −0.98 | −0.06 | −16 | 65 | −81 |
| V | VI | 0.16 | 6.26 | −0.70 | −0.01 | −4 | 101 | −105 |
| VI | VII | 81.6 | 22 | −1.05 | ~0 | ~0 | 550 | −550 |
| VI | VIII§ | ~5 | ~21 | .. | ~ −1.01 | −282 | .. | .. |
| VII | VIII§ | ~5 | ~21 | 0.000<br>±0.0005 | ~ −0.93 | −260 | −260 | .. |

† Data of Bridgman (1912, 1935, 1937), except where noted. Values of $\Delta H$, $\Delta E$, and $P\Delta V$ have been calculated by the present authors.

‡ Supercooled ice III.

§ Data of Brown and Whalley (1966) and Whalley *et al.* (1966).

Intuitively, it appears to the present author that the former of these two possibilities is the most likely. Thus, although indirect, it appears that the thermal anomalies in the properties of vicinal water lend support to a mixture model for bulk water.

## V COOPERATIVITY AND THE ENERGETICS OF SOME AQUEOUS SYSTEMS

### A Energetics of high pressure ice polymorphs

The small lattice energy differences between the various ice polymorphs have already been stressed in Section III.C.2.a. Table I (from the Monograph by Eisenberg and Kauzmann, 1969) shows some of the thermodynamic quantities for the transitions between various ice polymorphs. Note that the transitions between Ice-II and the types of ice adjacent to Ice-II in the phase diagram are characterized by relatively high entropy changes. The same applies to transitions involving Ice-VIII. For all other ice-ice transitions the entropy changes are far smaller (by a factor of about 10). These differences have been ascribed to ordering in the polar forms of ice (Ice-II and Ice-VIII). Ice-I has an entropy of approximately 0.8 e.u. (the Pauling calculation based on the disordered arrangement of the hydrogen atoms). Similarly, Ice-III, V, VI, and VII also possess disordered proton arrangements and hence, an additional extra entropy of 0.8 e.u. Thus, the entropy changes (around 0.8 to 1.2 e.u.) in transitions from either Ice-II or Ice-VIII to the other ice polymorphs are primarily a reflection of the change in entropy associated with the hydrogen disorder. Recall here the results obtained by Peschel and Adlfinger (1971). These authors deduced the differential excess molar entropy changes from their disjoining pressure measurements and found values ranging from $-2$ to $+2$ e.u. The variation from negative to positive entropies is not unique to the (liquid) water between the quartz plates: Thus, $\Delta S$ for the Ice-I to Ice-III transition range from about $-0.46$ e.u. (at $-60°C$) to about $+0.4$ e.u. at $-22°$. Thus, over the whole temperature range, a difference of 0.86 e.u. is obtained—roughly corresponding to that expected for disordering of the hydrogen atoms. It is not the intention here to attempt to demonstrate probable ice-like crystalline attributes of vicinal water. However, it is of interest to note both the notable magnitude of the differential molar excess entropies and those observed for the ice polymorphs as well as the change in sign. It would not seem unreasonable that changes in degree of proton ordering could occur in the vicinal water structures. Recall also that this may correspond to large differences in dielectric properties: Ice-I, III, V, VI, and VII have high dielectric constants (99 to approximately 193), while the proton ordered polymorphs have far lower values (3.66 for Ice-II; less than 3 for Ice-VIII, and approximately 4 for Ice-IX). Thus, the unusual dielectric properties reported by many authors for vicinal water appears entirely consistent with the entropy changes observed by Peschel and Adlfinger and corresponding changes in dielectric properties of the high pressure ice polymorphs. Furthermore, if the changes in sign of the differential molar excess entropies are indicative of changes in ordering of the protons in the vicinal water lattices, it is hardly surprising if notable changes are observed in the dielectric properties of vicinal water in general, and therefore, also for vicinal water in cellular systems (especially associated with biological membranes).

## B Ice/water interfacial phenomena (dynamical aspects)

### 1 *Freezing potentials*

Some of the most remarkable examples of cooperativity among water molecules have come from studies on ice and the ice/water interface. In this section, attention is directed to the structure and properties of water at the ice/water interface of a growing ice crystal.

In 1950, Workman and Reynolds described the generation of large potentials between an ice crystal (growing from a dilute aqueous electrolyte solution) and the unfrozen, supernatant solution. As a typical example, for a growth rate of about 10 microns per second, the potential for a $2 \cdot 10^{-5}$ (to $10^{-4}$) molar potassium chloride is of the order of 30 volts—the ice acquiring a negative potential. This potential is capable of sustaining a current in an external circuit. Under optimum conditions, the current may amount to some tenths of a microampere (per square centimeter ice formed). In the case of the potassium chloride solution (as indeed with all the alkali halides) the ice becomes negative with respect to the supernatant, unfrozen solution. As a current in an external circuit may be sustained as long as the freezing progresses, it is obvious that chloride ions are continuously taken in across the ice/water interface in the process of solidification while potassium ions are selectively rejected. Analysis of the ice after melting reveals that the dilute solution has become acidic and, conversely, the unfrozen solution has become partially alkaline (due to potassium hydroxide). A genuine charge separation process thus takes place at the ice/water interface. Ions are taken into the ice against a potential of about 30 volts. Workman assumed that the potential drop was essentially located right at the ice/water interface. The energy expenditure in taking up an ion against a 30-volt potential drop corresponds to 30 electron volts, or more than 700 kcal/gm mole ion incorporated! The fraction of ions incorporated may approach 98% of the available anions under optimum conditions (from a $2 \times 10^{-5}$ molar cesium fluoride solution). Such energetics are obviously "non-physical"—it would correspond to the energy of dissociation of fifty water molecules to hydrogen ions and hydroxide ions. The explanation of this mystery is undoubtedly that the potential drop is not immediately across the interface, but distributed over a finite distance, perhaps of the order of microns (or millimeters). The charges in the ice due to the halide ions are neutralized by protons moving relatively freely in the ice lattice. However, based on the idea that enormous energy changes could not be caused by a single molecular event, Workman conjectured that visible macroscopic, morphological changes might occur as the result of large-scale cooperative action and, hence, instigated a search by electron microscopy techniques for surface manifestations of this effect. As a result, Truby (1955) observed, by replicating the surface of ice single crystals, and hexagonal microstructure on the surface of ice crystals, frozen from pure water or very dilute solutions of cesium fluoride. The microstructure in the ice consists essentially of nearly parallel bundles of "microcrystalline entities" of about one to six microns in diameter, and about two to ten microns in length. Truby also found that the microstructure was more pronounced the lower the concentration of salt and, in fact, disappeared relatively rapidly with increase in ion concentration. Figure 15 shows a cross-section of a microcrystalline structural entity in ice formed in pure water, and Figure 16 shows (highly schematized) the relative surface coverage of microstructural entities as a function of concentration in solution, together with a curve showing the freezing potential as a function of concentration. It is seen that where the freezing potential reaches a maximum, the rate of disappearance of structure is also maximal (see Drost-Hansen, 1967). Furthermore, Truby was able to show that increasing the concentration of cesium fluoride from $10^{-5}$ molar to $5 \times 10^{-5}$ molar resulted in the reduction of the apparent step height from 500 Å to about 200 Å.

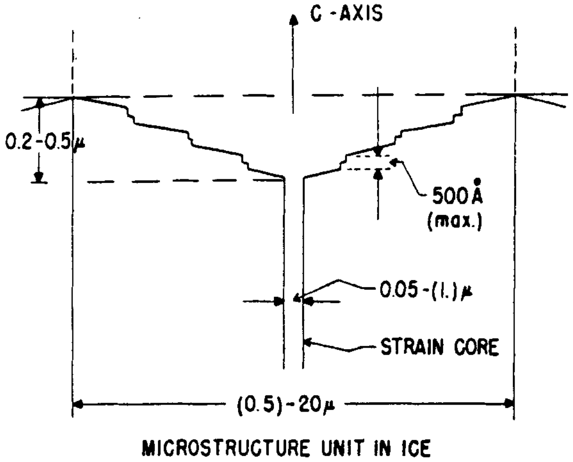

FIGURE 15 Schematic drawing of individual microstructural units in an ice single crystal. (Drost-Hansen, 1967.)

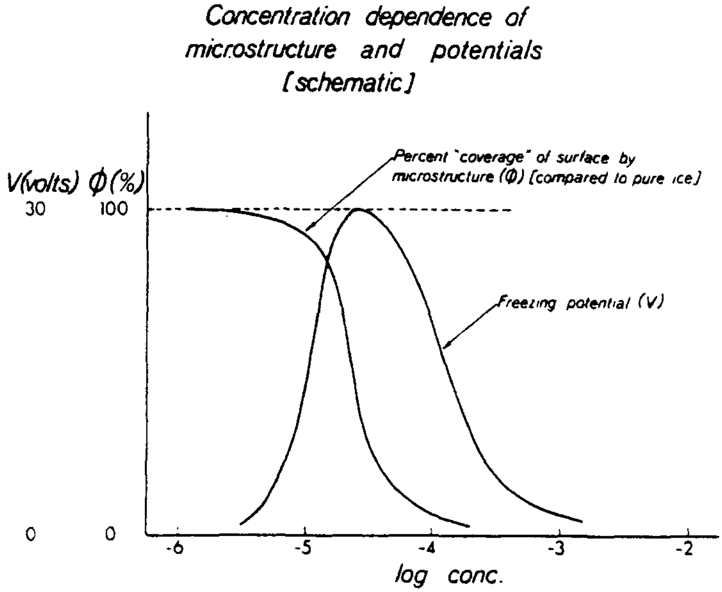

FIGURE 16 Freezing potentials and relative surface coverage by microstructure, as a function of electrolyte concentration. (Drost-Hansen, 1967.)

### 2 *Origin of microstructure in ice*

In 1967, the present author proposed a qualitative theory to explain the observed electrical and morphological phenomena. It was suggested that a lattice strain is built into a growing ice crystal because of the difference in bond energy between the vapor phase bond angle for water of 104.5° and the required, tetrahedral bond angle, 109°28′ in a "perfect" hexagonal lattice. For extremely pure water, this requirement for an "opening" of the bond angle by about 5° introduces a lattice strain energy. Previously, F. C. Frank (1951) had suggested that in crystals with large dislocation vectors, "strain cores" might be created. Truby (1955) had described how careful replication of aged, etched ice crystals revealed the existence of a void (seemingly, "infinitely deep") in the center of each microcrystalline entity. Gentile and the present author (1956) made some preliminary estimates of the likely diameter of the strain core in terms of assumed values for the interfacial ice/water free energy and for the elastic constants of the ice (which were taken to be identical with those of bulk, ordinary ice) and the dislocation vector which was taken to be $\frac{1}{2}$ the (multiple) step heights. The agreement between the observed and calculated values for the strain core was remarkable, although possibly fortuitous in view of the uncertainty surrounding each of the values used in the calculations (the Burger dislocation vector is normally only a few atomic or molecular diameters—not of the order of one hundred molecular diameters). As the concentration of electrolyte is increased, more and more ions become available for incorporation into the ice lattice and these ions serve somehow to reorganize the ice lattice locally to take up the lattice strain otherwise produced in the pure crystal.

Although Workman's initial suggestion now surely appears incorrect—namely, that the potential drop would be located essentially at the very interface of a growing ice crystal—this notion led to the important discovery of the microstructure! The freezing potentials themselves and their correlation with the microstructure can be explained only in terms of highly cooperative effects among several tens (or hundreds) of thousands of water molecules. It is interesting to estimate the quantities involved. If we assume the "radius" of an hexagonal microcrystalline prism may be approximated by the radius of an inscribed circle of about two microns and that the length of the hexagonal prism is five microns, the volume of each microcrystalline unit is $6 \cdot 10^{-11}$ cm$^3$, consisting of $2 \cdot 10^{12}$ water molecules. The number density (if *all* the ice is in the form of such microcrystalline entities) would be $3 \cdot 10^{11}$ hexagonal microcrystalline units per gram mole of water frozen. Compare this to the number of ions incorporated (assuming 100% incorporation of the fluoride ions) from a $2 \cdot 10^{-5}$ molar cesium fluoride solution, namely $2.2 \cdot 10^{17}$ fluoride ions per gram mole of water frozen (approximately $1 \cdot 10^5$ fluoride ions per microcrystalline unit). It is remarkable that this concentration of fluoride ions corresponds to the maximum rate at which the hexagonal microcrystalline structure disappears most rapidly! The calculations are obviously approximate in nature. Very rarely is the efficiency of ion incorporation 100% nor is the entire volume completely organized in the form of microcrystalline units. In fact, Truby referred to pure water as having 100% *relative* surface coverage, which actually might represent something like 60 or 70% of the surface covered with microcrystalline units (as detected by the electron microscope replicating technique employed). It should be recalled that even in the purest water attainable, there would always be a certain concentration of hydrogen and hydroxide ions and these could conceivably also serve to induce local "structural relaxation" and thus, lower the total number of structured entities found in the purest ice. Increasing the fluoride ion concentration from $10^{-5}$ to $5 \times 10^{-5}$ molar reduced the apparent step height from 500 to approximately 200 Å. This corresponds to approximately 100,000 water molecules being affected by the incorporation of *one* fluoride ion.

In this discussion of the freezing potentials and their origin, stress has been placed on the qualitative "reason" for the ion incorporation—namely, the lattice strain energies. The problem of the mechanism of "how" the ions are incorporated remains unsolved, in spite of a number of important studies (see, for instance, Gross, 1970; Jaccard and Levi, 1961; Pruppacher, *et al.*, 1968; Murphy, 1970; and others). The phenomenon is truly remarkable when one considers that the advancing ice front is associated with a negative potential which, intuitively, would have been expected to exert an attractive force on the cations—until a steady state distribution had been achieved, resulting in nearly identical incorporation rates of cations and anions. To overcome this difficulty, it was proposed (see Drost-Hansen, 1967) that the ice at the interface is not ordinary, hexagonal Ice-I, but rather is a polar form of ice. Specifically, it is suggested that freshly formed ice contains or consists of Ice-II (or another type of polar ice). The molecules in the interface of this polar, transient ice is oriented so as to present to the unfrozen, aqueous phase, a dipolar layer with the positive charges oriented toward the solution side. The anions in solution are "adsorbed" onto this positive surface layer, thus permitting the ice front to "overtake" the ions, while the positive ions ahead of the advancing ice front are continuously repelled. Work continues in the author's laboratory to test this hypothesis.

### *3 Ice densities*

Unexpected, independent, tentative confirmation of the existence of a polar ice has come from precision density measurements on freshly frozen ice made by Dantl and Gregora (1968). These authors measured the density of freshly frozen ice at constant temperature and observed a decrease of about 0.2% in density over a period of several hours; i.e., the initial density was larger than the equilibrium density. Certainly, this could not be explained in terms of inclusion of bubbles, and since the ice was frozen from reasonably pure water, could not be due either to inclusion of "brine pockets." It is proposed that the increased density reflects the transient occurrence of a polar, and hence more dense, ice. The density of Ice-II is about 1.15 grams per cubic centimeter. We intend to pursue the notion of Ice-II as a transient, high density intermediate by density measurements with a dilatometer constructed so as to make possible measurements of the density of a freshly formed "slush" of microcrystalline ice (formed by sudden cooling of highly dispersed water in a carefully supercooled hydrocarbon liquid). Other investigations of unusual properties of the intermediary layer of ice have been discussed in the paper by the present author (Drost-Hansen, 1967) and attempts to demonstrate the existence of polar ice at the interface by optical means also continue in this laboratory.

### *4. Cooperativity and polarized multilayers*

In summary, many of the properties of an advancing ice surface exhibit evidence for very large-scale cooperative processes among the water molecules. The numbers of water molecules involved may possibly range upwards in the millions. The energetics are characterized by very low values corresponding to very weak interactions as would be expected in the case of long-range cooperative phenomena.

Finally, it should be mentioned that the existence of polar ice at a freshly formed ice surface might constitute one possible example of a "polarized multilayer." The term "polarized multilayers" has been used by Ling (1962, 1969) in his treatment of the Induction-Association Hypothesis (based on a suggestion by Troshin). The present author wishes to stress that he feels that such "polarized multilayers" (if the semantics is indeed such as to mean "oriented, consecutive dipole layers") may occur at the ice/water interface, but are unlikely to occur over any significant distances in a biological system.

### 5. *Energetics of the microstructure*

As indicated in the discussion on the freezing potentials and microstructure in ice, it appears inescapable that large-scale cooperative effects occur at the water/ice interface. The energetics of this phenomenon are of interest. In general, the energy stored in dislocations in solids may amount to an appreciable fraction of the total surface energy of a crystal. Preliminary estimates (Drost-Hansen, 1967) suggest that this may also be the case for ice, although the contribution due to dislocations, per se, amounts only to 0.01% of the heat of fusion. The overt charge separation, effected during freezing of the ice, corresponds to a change in total lattice energy of about 0.2 calories per gram mole ice frozen (based on a current of about one microampere for a driving voltage of about 30 volts under optimum conditions of growth; rate of about 10 microns/second). In terms of the heat of fusion: 0.014%.

## C Energetics of disjointing pressure effects

We return briefly to the measurements by Peschel and Adlfinger (1971) of the thermodynamic properties of water between quartz plates as determined from disjoining pressure data. Figure 17 shows the differential molar free excess energies observed for water between two fully hydroxylated quartz plates separated 500 Å. The differential excess free energy is obtained by integration of the disjoining pressure. In this calculation, the density of the intervening water layers is, for want of any other information, assumed to be equal to that of the bulk water. (This latter assumption, no doubt, introduces a notable uncertainty. However, it is unlikely that the net result will be affected to the extent where the qualitative trends would be obscured). It is seen that the differential molar excess free energies vary between approximately 0 cal/g mole (at about 39–40°C) and as much as 6 cal/g mole. The differential excess molar free energy was used to obtain the differential molar excess entropies by differentiation of the analytical (power) expression for the

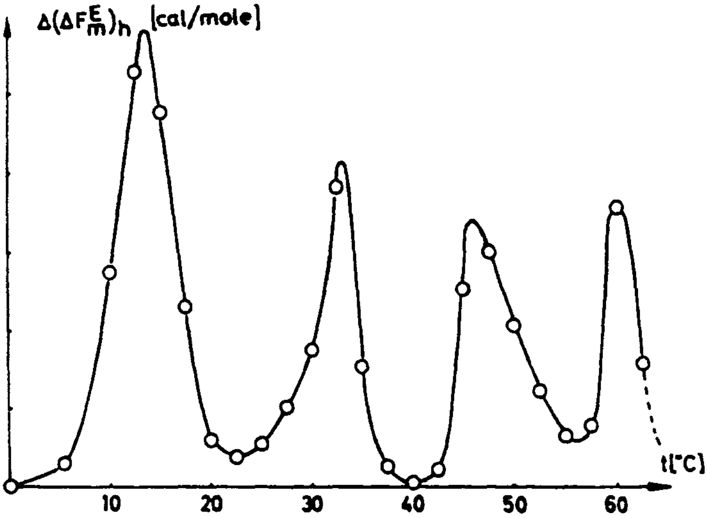

FIGURE 17 Differential molar free excess energy for water between quartz plates (500 Å layer thickness) as a function of temperature. (Peschel & Adlfinger, 1971.)

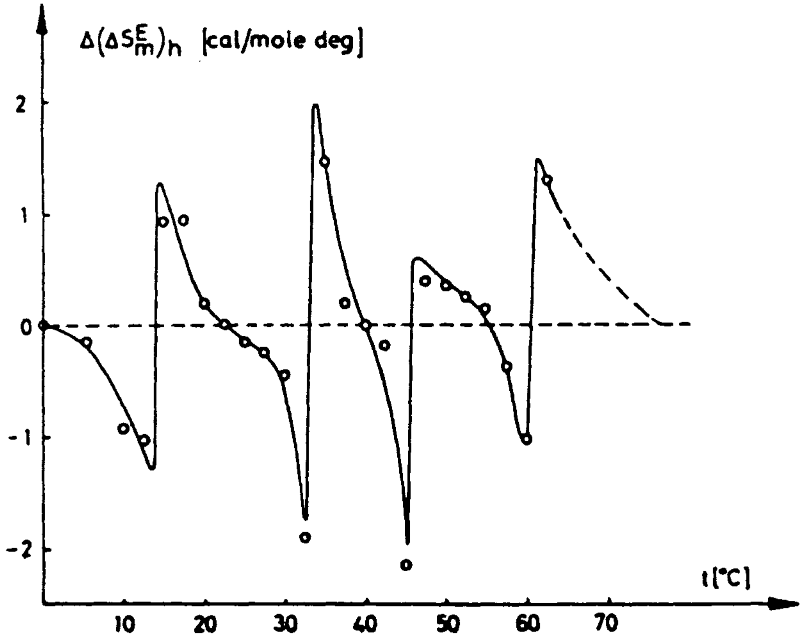

FIGURE 18 Differential molar excess entropy for water between quartz plates (500 Å layer thickness) as a function of temperature. (Peschel and Adlfinger, 1971.)

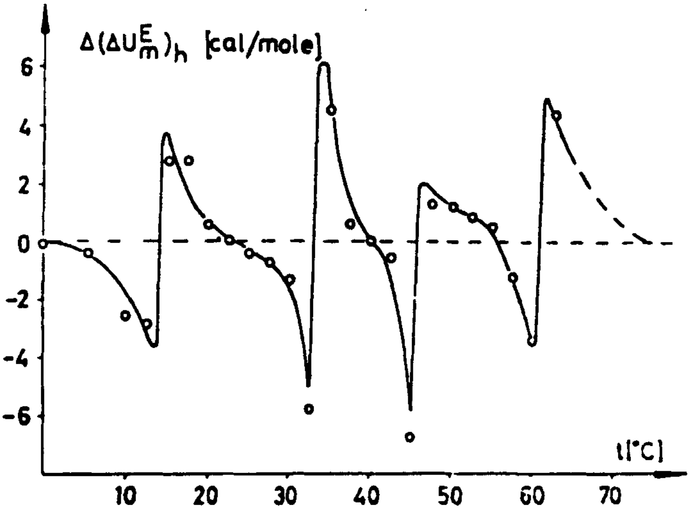

FIGURE 19 Differential molar total excess energy for water between quartz plates (500 Å layer thickness) as a function of temperature. (Peschel and Adlfinger, 1971.)

differential molar excess free energy. The result is shown in Figure 18. Finally, the differential total molar Helmholz energy is obtained by the equation:

$$\Delta(\Delta U^E_m)_h = \Delta(\Delta F^E_m)_h + T\Delta(\Delta S^E_m)_h$$

and the results shown (again for a plate separation of 500 Å) in Figure 19. It is seen that the Helmholz energy varies between approximately −6 and +6 cal/g mole.

For a plate separation of 100 Å, the differential excess energy obtained by the same method lies in the range between −33 and +30 cal/g mole. Peschel and Adlfinger note that the error may be as large as ±80%, but "nevertheless demonstrate the fact that the differential molar total excess energies are much less than thermal energy (*RT*)." This again is exactly the point stressed by the present author (Drost-Hansen, 1971) and we return to a discussion of this aspect in Section E.

## D Thermodynamics of adsorbed water (DTA studies)

Elsewhere (Section VII.D), the paper by Lumry and Rajender (1970) will be discussed in connection with the entropy-enthalpy compensation phenomenon. In the present context, attention is called to the statement by these authors regarding the necessary conditions for observing abrupt thermal anomalies. Thus, Lumry and Rajender note that "since in systems at constant pressure the occurrence of a sharp change in the slope of a parameter plotted as a function of temperature can occur only if there is a large entropy change, the kinks have been attributed to cooperative behavior of a number of water molecules. However, such behavior must reveal itself by heat-capacity spikes which have thus far defied detection." Presently, little evidence appears to have been published in the literature to suggest the existence of "heat capacity spikes." Some thermograms obtained on aqueous lipid systems do indeed reveal the type of thermal anomalies which would be indicative of cooperative, vicinal water structure changes. However, in almost none of the studies reported is it possible to draw inferences with any degree of certainty because of the intrinsic complexity of the lipids themselves (as mentioned elsewhere in this paper). For this reason, we have instead initiated some experiments aimed directly at determining if "heat capacity spikes" may occur in what we consider to be conceptually simpler, experimental circumstances. Mr. Chaur-Sun Ling and the present author have adopted what is essentially a "double-differential thermal analysis" approach. The method employed consists of DTA calorimetric measurements on porous Vycor samples. Two different experimental approaches have been used; in one type of experiments, thermograms are obtained with equal amounts of porous Vycor and equal amounts of water added (in each of the two cups of the DTA calorimeter cell). This should lead to a baseline showing neither exothermic nor endothermic trends if all conditions were completely equal. In our experiments, however, one sample (for instance, the reference) may consist of porous Vycor with an average pore diameter of 2500 Å, while the "unknown sample" may consist of an equal amount of porous Vycor, but with an average pore diameter of 200 Å. Obviously, the "microenvironments" of the two water samples are now different. Hence, if the larger surface-to-volume ratio system leads to increased structuring compared to the smaller surface-to-volume ratio system, the "heat capacity spikes" mentioned by Lumry and Rajender should be observed. Anomalies are indeed frequently seen. Unfortunately, the experiments, at this point, suffer from a notable lack of reproducibility; one complicating factor is the experimental uncertainty due to differering contributions to the thermogram caused by selective evaporation under slightly asymmetric conditions. In another series of experiments, samples of equal amounts of Vycor, but with same pore diameters, were again compared, but after exposure of the samples to different relative humidities (the total amounts of water adsorbed under these circumstances differ, but the differences are sufficiently small not to give a significant slope to the baseline). Again, in these cases, abrupt changes in slope as well as small specific peaks (endothermic peaks in the heating curves) are frequently observed. Unfortunately, these experiments also suffer from lack of reproducibility. The distinct changes in baseline slopes and baseline "offsets" which are usually observed in all of these experiments confirm that thermal anomalies do indeed occur in the properties of vicinal water. However, it remains to be seen if the apparent small endothermic peaks are indeed real. They correspond to 10 to 100 cal/g mole water if *all* the water in the sample contributes to the effect. This would then suggest that the transitions at the critical temperature regions may be first-order phase transitions and represent the latent heat of sudden "melting" of one type of vicinal, "solid-like" (?) ordered structure to yield another, ordered structure. However, in view of the experimental difficulties involved, it is also possible that the observed peaks are artifacts (perhaps due to rather notable specific heat changes) and that the transitions are indeed of second or higher order with no overt latent heat of transition. This point requires further careful experimental work for clarification, and such studies are underway in the author's laboratory.

## E Energetics of cooperative phenomena in aqueous systems

In the Table IV below are shown some characteristic energies for a number of structural processes involving water.

It is clear that the energies listed are very low compared to thermal energy at room temperature. Cooperative processes, characterized by extensive, long-range effects involving only small energy changes, are apparently characteristic of many hydration phenomena; as will be discussed below, this is perhaps particularly the case in biological systems. (It is obvious that,

TABLE IV

Energetics of various structural changes

| System | Reference | Energy diff. (cal/g mole) |
|---|---|---|
| Ice polymorph transitions | See- Kamb (1968)<br>Eisenberg & Kauzmann (1969)<br>Fletcher (1970) | 19 to 550 (mostly ~200) |
| Clathrates → ice | | a few hundred |
| Ice microstructural lattice strain effect | Drost-Hansen (1965) | 0.2 |
| Disjoining pressure effects | Peschel & Adlfinger (1971) | <35 |
| Transitions in water[a] adsorbed on vycor | Ling & Drost-Hansen (1972, unpublished) | 10 to 100 |
| For comparison: Heat of fusion of ice | See: Eisenberg & Kauzmann (1969) | 1440 |

[a] Minimum estimates, assuming the thermal effects correspond to a latent heat of a (first-order) phase-transition, involving *all* the water in the system.

in addition, highly energetic processes, involving water, also play a significant role in biological systems, especially the hydration of ionic and strongly polar groups. However, such systems will not be considered in any detail in the present paper.)

## VI THERMAL ANOMALIES IN BIOLOGICAL SYSTEMS

### A Specific examples

It is possible here to mention only a few examples of thermal anomalies in biologically interesting systems. Many more examples can be found in previous papers by the present author (see particularly 1971).

### *1 Viscosity of protoplasm*

Figure 20 shows the viscosity of protoplasm obtained by Heilbrunn (1924) from *Cumingia* eggs (*Cumingia* is a small mollusk). There appears little doubt that abrupt changes occur in the (apparent) viscosity of the protoplasm near 15 and near 30°C. In the absence of any other theory to explain such abrupt viscosity changes, it is reasonable to propose that the changes are manifestations of structural effects on the water of hydration of the solutes. It is obvious that the underlying molecular processes are likely exceedingly complex. Attention is drawn to the abruptness of the change—even more so than to the total, quantitative degree of change. The reason for stressing the abruptness is that presently many biological phenomena are interpreted in terms of protein denaturation or other (cooperative) processes due to con-

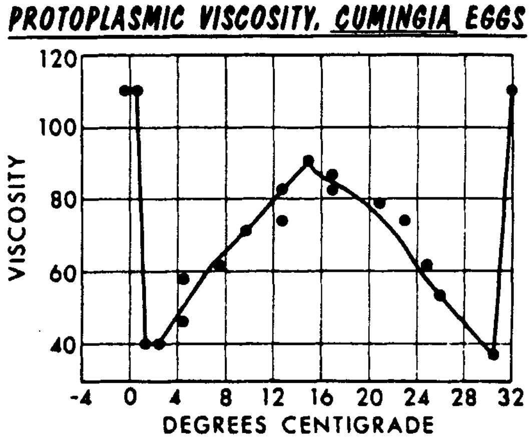

FIGURE 20 Viscosity of protoplasm from *cumingia* egg as a function of temperature. (Data by Heilbrunn; quoted by Johnson, Eyring and Polissar, 1954.)

formational changes in the constituent macromolecules. However, the degree of abruptness of change exemplified in the present case suggests that "normal" denaturation processes can hardly be expected to explain the experimental results. Note also that the temperatures of the anomalous changes coincide with the temperatures at which anomalous changes in vicinal properties of water in nonliving systems occur.

### *2 Rate of nerve impulses*

Figure 21 shows data obtained by Zotterman (1959) for the rate of discharge of nerve impulses in the lingual nerve from the cat. Again, some of the changes are highly abrupt and no current theory of neural processes are capable of predicting these changes. Note the marked minimum at approximately 16°, the rapid decrease between 25 and 35°, and the anomalous increase above 45° (the latter is the so-called "anomalous cold response"). In this figure, open circles are for cold receptors; filled circles for warm receptors.

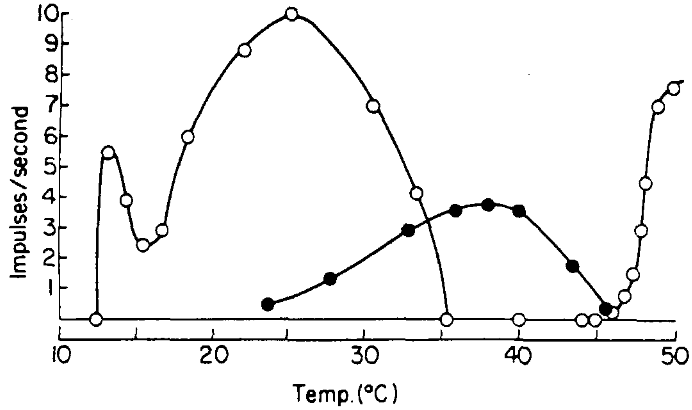

FIGURE 21 Frequency of discharge of lingual nerve from cat, as a function of temperature. (Zotterman, 1959.)

### *3 Rate of sodium transport across atrial wall*

Another example of temperature dependent anomalies in transport properties is shown in Figure 22. In this illustration is plotted the rate of diffusion of $^{24}Na$, (across the atrial wall from rat heart; in vitro) versus reciprocal absolute temperature, as reported by Chaudhry and Mishra (1969). Measurements were made under normal conditions as well as in the presence of ouabain. It is seen that distinct changes in the rates are observed at 15, 32 and 42°—where the change at 42° was observed only under "normal" conditions. (Note, however, that the curve segment indicated by the authors for the data

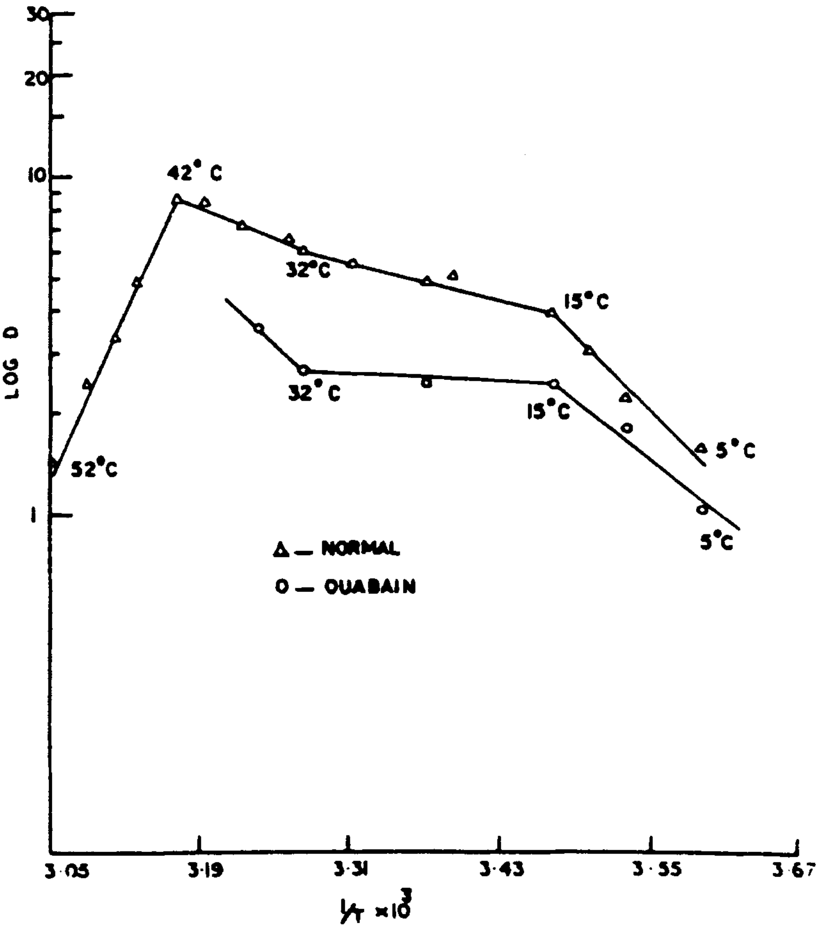

FIGURE 22 Arrhenius graph of diffusion of $^{24}$Na from rat heart. (Chaudhry and Mishra, 1969.)

obtained in the presence of ouabain is of little value since a straight line segment has been drawn through only two points!). The data do suggest that the transport of sodium across the atrial wall is not only strongly temperature dependent, but also suggest the existence of thermal anomalies in the transport process. Chaudhry and Mishra indicated that this phenomenon may be related to the structural changes proposed by the present author.

### 4 *Rate of chromosome abberation*

Wersuhn (1967) determined the rate of chromosome abberation as a function of temperature during microsporogenesis. The results obtained on the broad

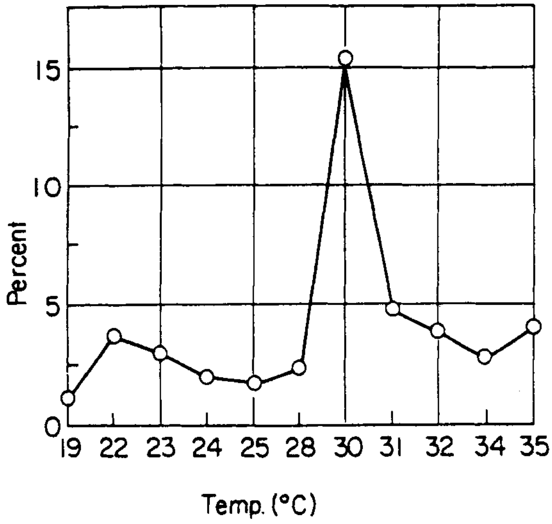

FIGURE 23 Rate (expressed as %) of chromosome abberation as a function of temperature; data of broad leaf bean (*Vicia faba*). (Wersuhn, 1967.)

leaf bean, *Vicia faba* are shown in Figure 23. Measurements were made at closely spaced intervals. An abnormally high rate of chromosome abberation (expressed in percent) was found near 30°. Wersuhn indicated that the data are statistically significant—the value at 30° is not merely a "statistical fluctuation." Furthermore, the results, according to Wersuhn, agree also with findings by Chira who also observed an increase in the "disturbance" of sporogenesis for *Taxus* near 30°.

In connection with the results by Wersuhn, compare the discussion of the increased entropy of surface formation near 30° as studied by the present author (1965); also, the anomalous increase in relaxation times for a water-filled quartz capillary as studied by Forslind and by Kerr and the present author, and finally the anomaly in the disjoining pressure at 30°. In these three cases it appears that a destabilization of vicinal water structure occurs around 30° (possibly accompanied by an increase in concentration of monomeric water molecules). In connection with the results by Wersuhn it is suggested that the anomalous chromosome abberation is the result of destabilization of the vicinal water. This finding maybe of considerable interest to the geneticist (see Drost-Hausen, 1969d).

### 5 *Respiration rate*

Figure 24 shows the respiration rate for the bat (*Artibeus*), as measured by Carpenter and Graham (1967). It is seen that the respiration rate changes from

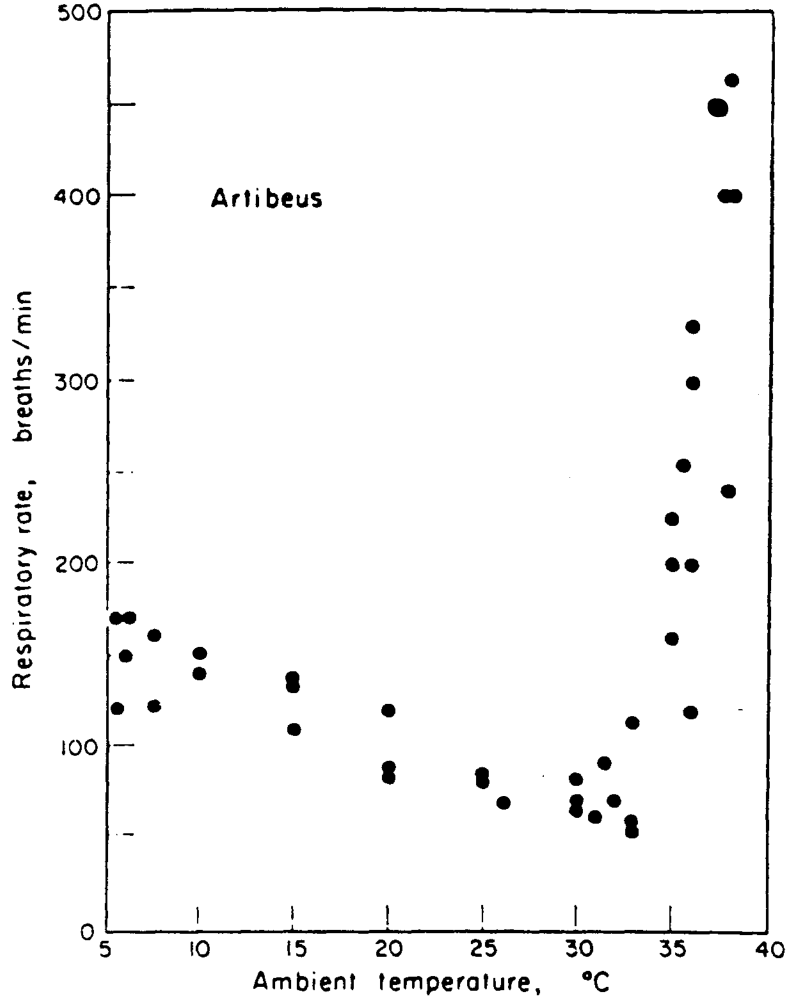

FIGURE 24 Respiration rate of bat (*Artibeus*) as a function of temperature. (Carpenter & Graham, 1967.)

a slightly negative to a highly positive temperature coefficient around 30°. Other measurements on the bat indicated that the heart rate likewise increases abruptly around 30–32°.

### 6 *Temperature optima and minima in biological systems*

The present author (1965, 1971) has proposed that body temperature for the mammals (centered around 37 to 38°) has been selected in the process of evolution as the optimum temperature—being approximately equally far away from 30 and 45°; since abrupt changes occur at these temperatures, it would undoubtedly have been difficult to devise feedback mechanisms whereby the thousands of simultaneous (independent as well as inter-dependent) reactions occurring in the living system could have been brought into cooperative action, considering that at the temperatures of the thermal anomalies drastic and often "qualitatively opposite" changes occur. Somewhat similarly, in the process of evolution, microorganisms have adapted to live in certain temperature intervals. This may be the basis for the major types of temperature responses reflected in the traditional classification of bacteria into cryophiles, mesophiles, and thermophiles—corresponding to the temperature ranges between consecutive thermal anomalies. Some microorganisms have, in the process of evolution, devised means whereby it became possible to grow optimally, for instance, between 22° and 25°, *and* between 35° and 40°—probably using different metabolic pathways (see Drost-Hansen and Oppenheimer, 1960). Note also that 60–62° is another temperature for which rapid changes are expected in biological systems on the basis of vicinal water structure changes and this temperature is the Pasteurization temperature. For a specific discussion of thermal death, see Section VIII.B.2.

The above examples are intended to illustrate that thermal anomalies are frequently observed in biological systems. The changes with temperature are often remarkably abrupt. Frequently (but obviously not invariably) the temperatures of the changes coincide with the temperatures for which anomalies are observed in the thermal properties of vicinal water in nonliving systems. Finally, note that for the present set of five examples—admittedly selected with prejudice for pedagogical reasons—only *one* mechanism need to be invoked to explain the vastly different thermal responses in these completely different biological systems (including data on a plant, isolated tissues [nerve and atrial wall], a mollusk and a mammal). No similar unification appears possible at this time, based on traditional biological thought. Obviously, the underlying unifying element suggested is the cooperativity of the hydrogen bonding of vicinal water.

### B Neglected aspects of thermal effects in biological systems

Abrupt changes in the thermal properties of biological systems have frequently been reported, but examples are readily available for which no evidence exists for such thermal anomalies. At least two reasons can be given for the absence of any manifestation of vicinal water structure. First, it is reasonable to assume that only those processes will be influenced for which the changes in energy are comparable to the energies of the water structures involved. Thus, for "high energy events" the detailed structure of the vicinal water may play no role whatever. Secondly, processes determined by small molecules in solution, particularly electrolytes, should not be expected to show any evidence of vicinal water structuring—at least not due to the nature of their own hydration structures. For solutions of biologically interesting macromolecules, the presence or absence of thermal anomalies must depend on the ability of the macromolecules to induce extensive water structures. This problem was studied by Glasel (1970a,b) and summarized in a recent paper by the present author (1971). Details of the temporal stability of the macromolecular conformations appear to play an important role in this connection.

Many examples can be seen in the current (as well as older) biological and biochemical literature of studies of temperature dependencies where trends in the data have been grossly ignored, mostly in favor of straight lines (such as in van't Hoff plots or Arrhenius plots). Most of these cases would not suffice as examples to prove the existence of thermal anomalies; however, once it is recognized (from some of the more substantial results—of which only a very small number have been quoted in this paper) that thermal anomalies are real, it becomes easy to see that alternative interpretations of various published data may indeed be called for. It is to be hoped that some of the future studies of temperature-dependent biological (and biochemical) phenomena will be carried out at far more closely spaced temperature intervals. (To facilitate such studies, different types of "polythermostats" have been constructed which conveniently allow for the simultaneous measurements of, say, two sets of experiments [such as a control for a "test" system] at each of 20 or 30 different constant temperatures, yielding data at incremental temperatures of, for instance, 1°C. At least two commercial versions of the polythermostat designed by Drost-Hansen and Oppenheimer [1960] are presently available).

The abrupt changes with temperature which are often manifested in biological systems and which appear to result from higher-order phase transitions obviously make quantitative treatments exceedingly difficult. Thus, as previously pointed out (Drost-Hansen, 1965), "logistics curves," for instance, for growth or development of an organism, may follow a theoretical expression with great fidelity between, say, 16° and 28°, but give completely discordant results slightly below 15°C or slightly above 30°C. By the same token, the concept of "degree-days," frequently used in various ecological studies, may completely lose meaning if temperature fluctuations occur which transgress one of the thermal stability limits.

## VII WATER IN BIOLOGICALLY AND BIOCHEMICALLY INTERESTING SYSTEMS

### A Classical view of the role of water in cell physiology

During the first third of this century, the concept of "bound water" in biological systems became generally accepted as an admittedly ill-defined but nonetheless very real attribute of cellular systems. During the late 30's and early 40's, physical chemistry of electrolyte solutions became increasingly frequently employed to describe cellular physiology on a molecular level. In these attempts, the ill-defined concept of bound water had to be dismissed. Soon the Debye-Hückel equation reigned unchallenged; water was water was water, inside cells and outside (at least within one or two molecular layers of an interface) and neither thermodynamics nor transport theories allowed for (nor could cope with) structuring of water near interfaces. Approaches to quantitative theories such as presented in *The Kinetic Basis of Molecular Biology* (Johnson, Eyring and Polissar, 1954) achieved the same degree of success in molecular biophysics as the Derjaguin–Landau–Verwey–Overbeek theory had achieved in the description of colloidal stability. Later, membrane phenomena were to be dealt with in the eloquent mathematical formulation of irreversible thermodynamics, and treatments of active transport flourished —despite the seemingly unending need for new, specific transmembrane carriers. By the middle 50's, practically no biophysicist had any obvious need for a detailed knowledge of the structure of bulk water itself and, least of all, for information about the structure of water near interfaces. The fact that substantial amounts of water are desperately needed for the helical stability of cellular macromolecules attracted but the most minute fraction of attention, compared to the intensive study of mechanisms of gene action and protein synthesis. As recently as 1968, Cole discussed in 550 pages, *Membranes, Ions and Impulses*, without once indicating a possible, unique role of water near interfaces, nor even showing an entry for the word "water" in the index to this monumental text. Szent–Györgyi (1971) summed up the state of affairs concisely: "biology has forgotten water, or never discovered it. According to Sir Oliver Lodge, the last thing a deep-sea fish would discover is water."

A new era in the study of water in biological systems was initiated by Jacobsen (1953, 1955), Szent–Györgyi (1957), and Klotz (1958). These authors re-introduced the notion that cellular systems might contain structurally modified water. Thus, Szent–Györgyi (1957) stated: "the formation of such water structures should not be confused with the old idea of 'bound water.' 'Binding' involves rather the idea of energy than that of structure. 'Binding' means a certain force, energy needed to remove a molecule from its site. Such 'bound' molecules, having their dipole forces engaged, are also unfit to serve as solvents for other solutes. Such a binding is especially strong around free charges, as those of ions. The order thus produced is 'short-range order,' the number of more firmly held layers of molecules being very small, 1–2. Contrary to this the building of lattices means 'long-range order' in which the single molecules collaborate collectively." The last sentence of the above quote may well be taken as the starting point of the new era of water structure studies in cellular systems—an era which by 1972, is just barely beginning.

### B Implications of thermal anomalies and sites of action of water in biological systems

Many abrupt changes in biological systems over narrow temperature ranges probably reflect the underlying changes in the vicinal water structure of the biological systems. As discussed elsewhere and mentioned briefly in the present paper, other processes do give rise to rapid changes over narrow temperature intervals—independent of water structure (such as lipid/lipid transformations, which may occur in completely anhydrous phases). However, in general, it appears that water structures are indeed frequently implicated, if not the controlling factor. Again, it is stressed that the water structure effects may be superimposed on conformational changes dicated purely by the statistical mechanics of the macromolecular chain stabilities. However, in the process of evolution, it is likely that these superimposed constraints may have influenced the selection of the "optimal" biomolecules of functional importance. The exact sites of the water structure changes in the biological systems have obviously not been established, but it seems highly likely that macromolecular hydration shells are involved. The influence of the vicinal water on membrane processes must also be considered.

The remarkably high surface-to-volume ratio of biological systems was illustrated by Robinson (1970) who noted that in man, approximately two thirds of the body weight is water—approximately 23 liters of intracellular water is divided into $10^{14}$ separate compartments, each separated by a membrane (and by extra-cellular fluid with notably different composition). Note that this corresponds to the equivalent of $8 \times 10^{12}$ water molecules per "compartment." Admitting to gross oversimplification, this corresponds to spherical "globules" of water with a radius of $13 \times 10^3$ water molecules.

#### *1 Water/membrane systems*

A review of the properties and structure of water in and adjacent to membranes has been presented recently by the present author (1971, loco cit., pp. 87–107). Only two examples will be mentioned briefly in this paper. Coster and Simons (1970) have studied the dielectric properties of water near a lipid membrane (bimolecular leaflet membrane). The result from the study indicated that the water layers adjacent to the membrane were affected over distances of at least 0.1 microns and possibly as much as 4 microns. The authors called attention to the need to examine carefully some of the underlying assumptions which were required in order to obtain these estimated values for the thicknesses of the modified water layers from the experimental data. However, an inspection of their treatment has failed to reveal any obvious flaws, at least to the present author (see, however, the comment by Schwan, Section III.C.3). It is of interest to recall also that very thick layers of structurally affected water were postulated by Schulman and Teorell from a study of what might be referred to as a "half a bimolecular leaflet"—namely, for water beneath a monolayer at an air/water interface (see Section IV.D.5c). The present author finds it difficult to accept that water should be affected to a depth corresponding to a hundred thousand molecular diameters, but it is interesting that Blank and LaMer (1957, 1958) have confirmed that very thick water layers are induced to move together with a monolayer of fatty acids on water.

### *2 Water/macromolecular interfaces*

In biological systems, specific water structures may influence the overall properties and functioning via a large number of different sites. The processes involved in metabolism, respiration, or the functioning of muscles or nerves are typical examples. Ultimately, most of these processes can be viewed in terms of the macromolecules involved: polysaccharides, polypeptides, polynucleotides, proteins in general and specifically, enzymes. A large amount of the evidence for the existence of structured vicinal water adjacent to many biologically important macromolecules has been discussed in a recent paper by the present author (1971). It is emphasized again that the presence of any interface appears to stabilize water structures over distances of about 0.1 micron. Szent-Györgyi (1971) has observed "Physics, on the whole, has not paid much attention to these water structures, probably because their thicknesses, as compared with macroscopic dimensions, is rather small. We biologists are interested in cells, and cells are small and the distances between their morphological units are still smaller, and so these water structures may play a decisive role in biological functioning."

*a Monolayers and lipids* Monolayers have served as "inspirational models" for the behavior of lipids in considerations of biological membranes, although Chapman and Wallach (1968) have called attention to the inherent dangers in overgeneralizations of this type. However, the problem itself of monolayer behavior and particularly the extent and depth to which the aqueous substrate beneath the monolayer is influenced, is of intrinsic interest —apart from possible generalizations to biological membrane models. Hence, in this section we discuss some aspects of aqueous interfacial phenomena in the presence of partially (or completely) monolayer-covered water surfaces, but mention only briefly a few observations regarding water/lipid systems in general.

Attention is first called to two aspects of vicinal water structuring which may be important. In a recent paper (Drost-Hansen, 1972), the present author stressed that the structure of water beneath a partial (or complete) monolayer at the air/water interface may be extensively structured—similar to the structuring encountered for water near solid surfaces. (The reader is referred to the paper for some of the experimental evidence on which this suggestion is based). We present here only two examples. Phillips and Chapman (1968) studied the monolayer characteristics of myristic acid on a $10^{-2}$ N HCl solution. Figure 25 shows the entropy and heat changes calculated from the data by Phillips and Chapman as a function of temperature. It is seen that for a surface area of the acid of 24 Å²/molecule, both the entropy and the heat of condensation changes slope abruptly at 15°. It is suggested that this change owes its origin to the structural changes in the aqueous subsurface layers; note that the transition is quite sharp.

A point of clarification may be appropriate here regarding "two-dimensional" second-order phase transitions. Landau and Lifshitz (1958) discuss the problem briefly, based on the Onsager treatment. The point is made that from the Onsager theory one would expect a logarithmic singularity, such as represented in the expression for the thermodynamic potential, $\phi$ (as a function of temperature) near the transition point.

$$\phi = a + b(T - T_c)^2 . \log|T - T_c|$$

This leads to an infinity in the specific heat near the transition temperature,

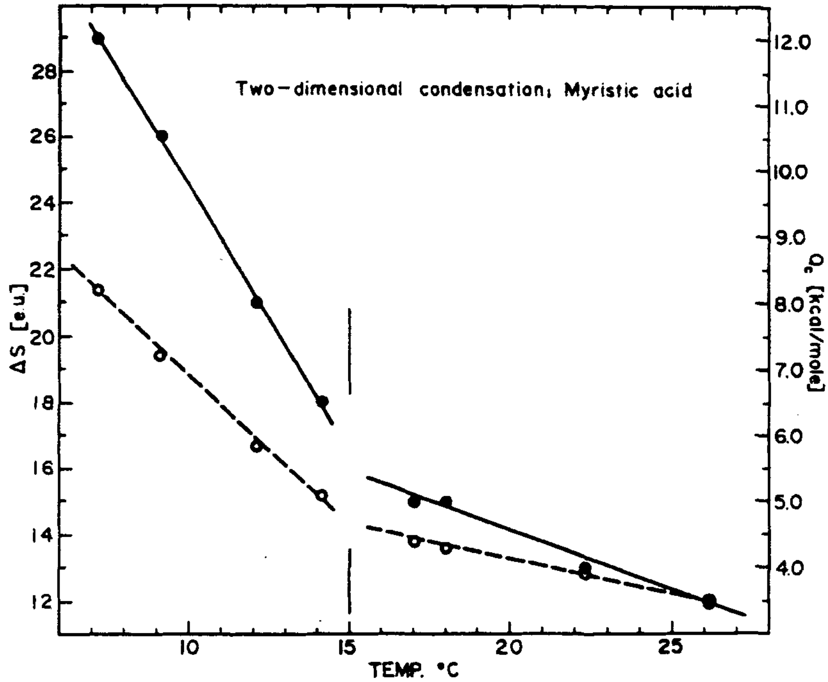

FIGURE 25 Entropy and heat changes for myristic acid on $10^{-2}$ N HCl solution. (Data by Phillips and Chapman, 1968.)

$T_c$. It leads, incidentally, also to the conclusion that the "pressure" (i.e., the surface pressure) affects the transition temperature. In other words, a transition point for a "similar lattice" in three dimensions would be different from that for a two-dimensional state, and in addition, depend on the surface pressure. Finally, according to Yang (C. N. Yang, 1952), this leads to a very weak dependence of the order, $\eta$, the difference between the critical temperature and the actual temperature, namely of the form

$$\eta = \text{const}(T_c - T)^{1/8}$$

As a result of these considerations, and some additional consideration of changes in symmetry between the phases above and below a transition point, Landau and Lifshitz conclude second-order transitions are possible only between phases of different symmetry. By second-order transitions, we again mean transitions with a continuous change of state of the system (surface film). However, as opposed to the three-dimensional case, it turns out that one cannot assert that the first derivatives of the thermodynamic quantities (such as the compressibility, coefficient of thermal expansion, etc.) undergo a finite discontinuity at the transition point, but have a logarithmic singularity.

The above considerations are, of course, valid, but the point must be made in the present context that for the specific case of film-forming materials on aqueous substrates, the above analysis is not strictly (and possibly not at all) applicable and this problem is of immediate interest to biological systems in connection with the interfacial film behavior of, for example, lipoproteins on aqueous substrates. The reason for the inapplicability of the straightforward consideration discussed above is that the systems are not truly two-dimensional. This point may be illustrated by an example from measurements by Enever and Pilpel (1967), shown in Figure 26. A transition occurs in the surface viscosity of stearic acid on dilute calcium chloride solutions around 15 (to 21)°C. The point is that the interactions are *not* only those between the constituent molecules of the two-dimensional surface film. On the contrary, in view of the discussion in the present paper (see also particularly, Drost-Hansen, 1972), the interactions include the interactions between the associated water molecules in the aqueous subsurface layers. These subsurface layers, in turn, may be of considerable depth. Even if they were merely one or two molecular layers in depth, their contribution would certainly change the detailed, geometrical description from the two-dimensional film phenomenon to a "thin, three-dimensional" phase problem. Furthermore, as emphasized here, the depth of the affected water structures may be extensive. While the present author does not subscribe to the claim, for instance, by Schulman and Teorell (1938) (see also Pak and Gershfeld, 1967, however) that the water under a layer of oleic acid may be affected to a depth of 30 microns, he is certainly willing to believe that some

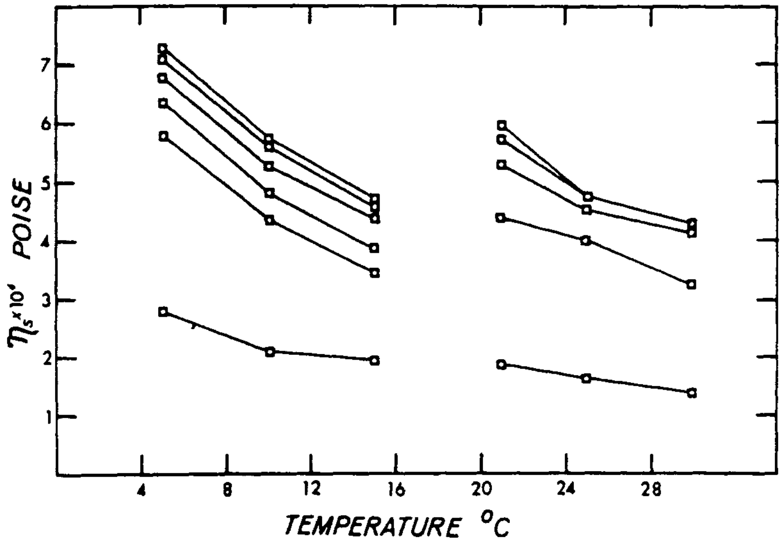

FIGURE 26 Relative surface viscosity (in surface poises) for stearic acid on 0.5 millimolar calcium chloride solution as a function of temperature. (Different curves for different ages of the surface films; starting from the bottom). (Enever & Pilpel, 1967.)

surface films affect the substrate structure to a depth of fractions of a micron. Thus, the problem is not truly of the nature of a strictly two-dimensional film, but rather of an almost "bulk, three-dimensional" state of aggregation. This type of consideration applies as well to the studies by Trapeznikov (see Drost-Hansen, 1965, 1972) and to many of the classical studies of surface film phenomena, as studied, for instance, by the Langmuir techniques by innumerable investigators. The above argument is not meant to deny that a truly two-dimensional film (such as argon adsorbed on mercury) may exhibit the properties predicted from the statistical mechanical considerations based on the treatments by Onsager, Yang and others.

The present state of understanding of the biophysical role of lipids (as opposed to the biochemical role) in biological systems is best characterized as *exceedingly complicated.* For two recent discussions of this aspect, see, for instance, the articles by Träuble and Haynes (1971) and Salsbury, Darke and Chapman (loco cit., p. 9). The latter article is particularly concerned with water/lipid interactions in biological systems. Chapman especially has, over the years, contributed some of the most significant information regarding water/lipid behavior in biological systems. Notwithstanding the types of careful studies mentioned above, the present state of affairs resembles a giant puzzle, of which only a minor fraction of the pieces are in their correct places and no one knows if some of the key pieces are missing. The problem in no small way stems from the "continuing ooze of data that results from putting phospholipids in this state and that into various expensive machines." (Anonymous note on phospholipids in *Nature*).

Obviously, a review of this muddle of information is not possible in the present article. Hence, referred to below are only a few select examples where water/lipid systems appear to reflect what may be the manifestations of the water structure changes at the characteristic temperatures discussed in this paper. Once again, it is stressed that pure anhydrous lipids often show abrupt thermal anomalies and that mixtures of various lipids (cholesterol and lecithin, for instance) are even more prone to reveal highly abrupt, unusual temperature dependent properties. It is merely suggested here that superimposed on these phenomena may be the discrete effects of water structure.

An example of such superposition of effects may possibly be seen in the data reported by Chandan *et al.* (1971). These authors studied the nature of fat globule membranes and constituents obtained from milk by DTA. In the presence of 32.5 and 7.5% water, notable endothermic peaks were observed at 15 and 44°. Upon drying the material over phosphorus pentoxide, the endothermic peaks moved, respectively, to 11 and 42°. Unfortunately, it is not possible to decide the extent to which the treatment with $P_2O_5$ did indeed remove all the water. Extraction of the lipids with acetone or chloroform-methanol gave different DTA curves and redispersion of the extracted material did not seem to reconstitute the material to the degree where the original thermograms were reproduced. Chandan *et al.* concluded that the major transitions are due to the lipid components and the transition between 40 and 44° was specifically attributed to melting of triglycerides (primarily on the basis of the large heat absorption involved). It is interesting to note, however, that for washed cream, transitions were also obtained at approximately 15 and 30°. See also the article by Ladbrooke and Chapman (1969).

As pointed out by Träuble and Haynes, "the exact nature of the structural changes at the transition temperature is not well known" for instance, in the case of the dipalmitoyl-phosphatidyl-choline-water system. It is of interest to quote these authors on this topic: "there is evidence that the polar head groups have a higher degree of rotational freedom above the transition point. It can be further concluded from calorimetric, x-ray, infra-red and nuclear magnetic resonance studies that the hydrocarbon chains are in ordered, quasi-crystalline arrangements, below the transition temperature, while above the transition point they are in a 'more fluid' state with considerable internal motion." The authors go on to point out that little agreement exists regarding the extent of the fluidity (of the hydrocarbon chain) in the liquid crystalline state. Some workers have postulated that the chains are liquid-like and highly disordered with considerable flexing, coiling and twisting of the chains. In this model, the structural changes at the transition point are characterized as a "melting" of the hydrocarbon chains. Other workers postulated the hydrocarbon chains are relatively ordered, and have a limited degree of rotational freedom, corresponding to that in a condensed monolayer. In this connection, compare the discussion by the present author (1971) of the likely *mutual* interactions between many macromolecules, and the vicinal water structures: it appears, for instance, that many polyelectrolytes will impress certain structural characteristics on the water adjacent to these molecules, but conversely, the cooperative, low-energy vicinal water structures may impose conformational restrains on the macromolecules (see Drost-Hansen, 1971).

In the article by Träuble and Haynes is indicated an interesting and rather abrupt anomally at approximately 44°C. As is the case in the discussion of the anomalies in enzyme kinetics, some of the reasons for believing that the water structure changes may play a significant role is based on the coincidence of temperatures observed for the anomalies under consideration and the now fairly well established anomalies in water near such "inert and well defined" surfaces as quartz, glass or many types of synthetic membrane materials. The coincidence of the temperatures of the thermal anomalies could represent nothing more than "numerology"; but the frequent occurrence of close correlations between the temperatures lends credence to the assumption that the water does indeed play an important role.

For another example of very discrete, abrupt changes in water/lipid interactions, see the recent paper by Salsbury, Darke and Chapman (loco cit.). The article by Salsbury *et al.* is also of interest because of its discussion of the role of the water. We quote: "The first layer of water may therefore be considered as adsorbed, bound and partially oriented, in that the OD bond direction is maintained to a limited degree, but with rapid deuteron jumping taking place. A simple model for the localized water might be rapid reorientation about axes perpendicular to the absorbing 'plane,' but slow orientation about the axes parallel to the plane." The authors go on to observe "the partially oriented water will bridge the opposing layers of lipid head groups in a bilayer model. With increasing amounts of water, the additional layers of water may be stabilized by the head groups to decreasing extents." The latter is conceptually similar to the proposal by the present author of vicinal stabilization, decreasing as a function of distance from the "solid" interface. Obviously, the present author is suggesting that *extensive* depth of ordering may occur—a situation which is not likely to be realized in the particular systems described by Salsbury *et al.* because of the relatively high lipid-to-water ratio.

*b Polypeptides and nucleic acids* Cooperative phase transitions in polypeptides are attracting increasing attention. Much of this work is associated with specific phase transitions due to complex formation. However, it appears that a number of results have been reported in the literature which suggest that straightforward stoichiometric relations cannot explain all observed results on the polypeptide mixtures. Thus, in a recent article, Davies and Davidson (1971) have shown some highly unusual temperature dependencies for melting profiles of selected polypeptide mixtures. The authors observed that "the melting curves are not typical of a normal cooperative transition, but suggest that some intermediate complex may be involved in the dissociation process. This is puzzling, since the binding isotherm at 5°C is quite sharp and shows no sign of a multistep binding process." Figure 27 shows the melting profile for a 2:1 poly a-3-methylxanthine complex as a function of temperature, while Figure 28 shows other melting profiles at different stoichiometric ratios. Other interesting temperature effects on adenylyl adenosine were reported at the same time by Kreishman and Chan (1971).

Recently, Schleich *et al.* (1972) have studied the influence of temperature and sodium perchlorate concentration on conformation of uracil nucleosides and nucleotides in aqueous solutions by NMR measurements. The authors first briefly review the "traditional ionic hierarchy" of effects of salts on the conformation of biopolymer stability, for instance, the conformity to the Hofmeister series. The authors note that the effects of the ions can be either direct—through direct binding of an ion to a portion of a macromolecule—or

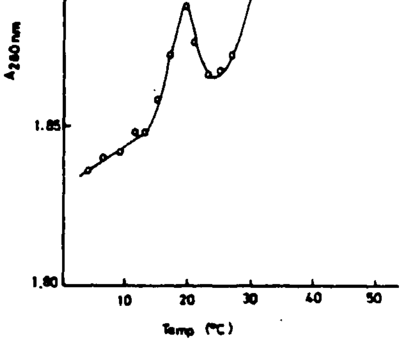

FIGURE 27 Melting profile for 2 : 1 poly a-3-methylxanthine complex. (Davies and Davidson, 1971.)

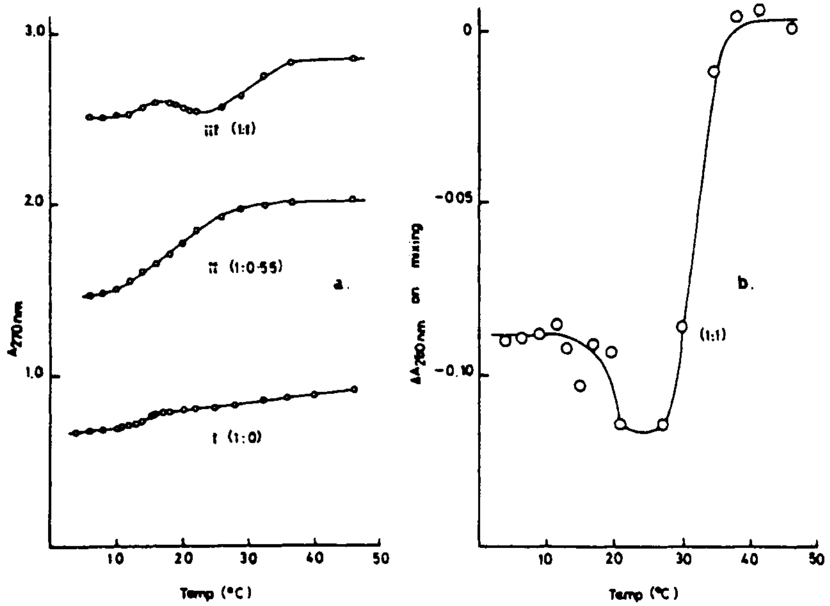

FIGURE 28 (a) Melting profiles for poly a-3-methylxanthine mixtures at pH 6. Ratios of poly a to 3-methylxanthine indicated on each curve. (b) Hypochromicity (at 2,800 Å) as a function of temperature of poly a and 3-methylxanthine in solution (ratio 1 : 1.62). (Davies and Davidson, 1971.)

by an indirect mechanism—via the ion induced changes in the water structure, which "in turn, modulate the energetics of some macromolecular stabilizing interaction, such as hydrophobic interaction of apolar moieties." The authors go on to note that a 2 molar sodium perchlorate solution at 25°C has the same effect on the water structure as raising the temperature of pure water to 60° (based on earlier work by Schleich and von Hippel, also using an NMR approach). (See, in addition, von Hippel and Schleich, 1969). In the recent paper, Schleich *et al.* (1972) conclude that the parallelism between increased temperature and increased salt concentration is not invariably obvious. Thus, from analysis of coupling constant data, it is found that temperature may produce changes opposite to those induced by increasing sodium chloride concentration; the disruption of the water structure due to the ions is not equivalent to the disruption by the addition of neutral salt denaturants. Hence, the authors conclude "if one believes in the validity of the liquid structure temperature concept (Bernal and Fowler, 1933) then the conclusion must be reached that *simple* water structure effects are not operating in the definition of nucleoside and nucleotide ribose conformations." In turn, this leads the authors to conclude that water structure is not a principal determinant in molecular conformation of these compounds. The present author suggests that an alternate interpretation would be to distinguish the effects of the salts on the bulk water structure as compared to the effects on the vicinal water structure. The latter, of course, is rarely studied except possibly inadvertently in infra-red studies (in both fundamental and overtone bands) using very thin cells where wall effects may play a role.

The examples referred to here from studies on polypeptides are of considerable importance. Lifson (1971) has emphatically pointed out that polypeptides—like other, one-dimensional chains—do not exhibit a true phase transition, but rather undergo changes over a temperature range of "10–20°C in favorable cases." Compare this range with the abruptness of the changes indicated for the thermal transitions observed by Davies and Davidson (1971a,b). The present author strongly feels that the sharpness frequently observed is good evidence that some of the transitions seen in the polypeptides are due to order-disorder phenomena in three dimensions: in other words, due to the conformational constraints imposed by the spatially extended vicinal water structures. The present author does not take issue with the statement by Rice (1971) that helix-coil transformations may give a rather good picture of such transitions, using only short range interactions; i.e., it is agreed that it may not be necessary to invoke long range forces to explain the basic features of helix-coil transitions. However, the examples discussed here do suggest that superimposed on the characteristics implied in the general theory of helix-coil transformation, some type of long-range effects are needed which may give rise to abrupt changes of the type shown in Figures 27 and 28. Hijmans (1971) has addressed himself to this as he posed the question, "should one, therefore, not expect that the driving force for the formation of helices originates at least partly in the solvent? One could, for instance, imagine that the presence of DNA strands would cause disturbances in some kind of local ordering in the solvent, which may be reduced by combining pairs of strands into helices."

"Bound water" in biological systems was, in the past, frequently determined as either water which did not act as "normal solvent," or as the water which did not freeze at a certain temperature below 0°C. Bakradze *et al.* (1971) have attempted to make use of the non-freezing water in a quantitative fashion for determining the amount of hydration of tRNA. The amount of "bound water" was calculated as the deficit of heat of fusion of ice in calorimetric measurements in the region of the phase transition. Unfortunately, the qualitative results obtained from the initial experiments by Bakradze *et al.* are unable to yield any specific information with respect to the nature of the water of hydration of the tRNA. A large fraction of this water was ascribed to the binding of six water molecules per each phosphate group (as suggested in the literature) while another fraction is contributed to hydrophobic bonding due to the non-polar surface groups. The authors point out that likely the water of hydration of tRNA does not cover the surface of the macromolecule as a uniform monolayer, but rather that discrete water aggregates may occur on the surface. Not only need the water not be uniformly distributed over the surface of the polymere but large amounts of water are found associated with nucleotides, not participating in stablizing the helical portion of the molecule. Compare the studies by Kuntz (see 1971a,b), who concluded that, while the major part of the water in polypeptides is likely associated with ionic groups, 25 to 30% may be "bound" by non-ionic groups.

Another example suggesting that the vicinal water structure may possibly play a crucial and perhaps dominant role may be seen in the study by Rimai *et al.* (1970) of the quasi-elastic scattering from RNA-ase solutions. The temperature dependence for the apparent diffusion constant as well as the effective apparent size shows extrema at around 45°. Viscosity anomalies are also frequently observed: see, for instance, the anomalies around 30 and near 60° observed by Grant *et al.* (1972). on $(\mathrm{dl} - \mathrm{dC})_n \cdot (\mathrm{dl} - \mathrm{dC})_n$ solutions. As stressed before, derivative curves with respect to temperature, are likely to yield considerably more useful information than has been obtained in the past. Thus, derivative melting profiles have been obtained by Marushige and Bonner (1971), for various chromatin fractions. These curves are definitely rather complex and appear to be the superposition of a number of distinct, discrete maxima. For other abrupt changes, see for instance, the article by Stenesh *et al.* (1971) on phenylalanine incorporation by various forms of *Bacillus licheniformis* and *Bacillus stearothermophilus*. Very sharp changes are observed near 45 and 60°.

For other interesting examples of rather abrupt and unusual temperature dependencies in polypeptide systems, see also the more recent article by Davies and Davidson (1971b). These authors studied base pairing equilibria between some polymers and their complimentary monomers as a function of temperature. See also the article by Pörschke (1971) on the thermodynamics of the helix-coil transition of oligoadenylic + oligouridylic acids. Unusual temperature dependencies have also been obtained by Small and Peticolas (1971) by Raman scattering data on various polynucleotides. A large number of additional examples could be added to those mentioned above. In calling the attention of the reader to these studies the reader is admonished to keep in mind that systematic trends in the data are often overlooked or ignored by the various authors. Hence, it is proposed that in any re-examination of papers dealing with temperature effects on biopolymers, the possible superposition of transitions due to the vicinal water structures be kept in mind.

Finally, see the article by Dean and Lebowitz (1971) on the formaldehyde thermal transitions of DNA. Again, the curves observed are frequently very complex—certainly not readily interpretable in terms of a single denaturation mechanism. For other examples of details in what is normally considered routine melting curves, see the article by Flamm *et al.* (1967) on isolated strands of DNA.

*c Proteins* Among the proteins, collagen enjoys a unique role biologically as the major constituent of vertebrate connective tissue, and biochemically as one of the best described, simpler macromolecules. A large amount of work has been done on the hydration of collagen as well as on the heat-denatured, soluble "gelatin." Collagen appears to possess hydration structures which reflect vicinal water structuring. For hydration studies in general, see the extensive writing of Berendsen and co-workers (see 1967, for references). Collagen is one of the few proteins known to contain significant amounts of proline, 3-hydroxyproline (in small amounts) and 4-hydroxyproline. This is likely important in connection with studies of vicinal water structuring in view of the dielectric measurements by Shepherd and Grant (1968a,b); these authors observed the relatively abrupt disappearance of a dielectrically distinguishable type of water of hydration, respectively between 30 and 40°C, and 20 and 30°C (see Section IV,B.5) for 1 molar aqueous proline and 1 molar hydroxyproline.

For an example of what appears to be a thermal anomaly in the properties of the vicinal water of collagen, see the comments by the present author (1971) based on the data by Berendsen and Migchelsen (1966). A good deal of additional evidence for the role of vicinal water structuring has been collected by the present author (unpublished data based on literature searches); only a few examples will be discussed.

French, Angus and Walton (1971) have studied the properties of collagen solutions, using an "optical self-beat spectroscopy" (OSB) method. In this approach, a spectral analysis is made of the Raleigh-scattered radiation from the macromolecular solutions. The spectrum observed is essentially the result of the Doppler shift of the scattered radiation which results from (random) translational, rotational and flexing motions of the individual macromolecules. Using the OSB spectroscopic approach, the half-width of the Raleigh scattering was determined (in Hz) as a function of temperature on a concentrated gelatin solution. The results are shown in Figure 29, where the half-width is shown as a function of temperature of solution for both decreasing and increasing temperatures. Note the significant increase in half-width in the interval between approximately 26 and 37° with a relatively sharp peak near 30 to 32°C. The shape of this curve resembles the half-life of vibrations in the "ultra-low" relaxation measurements by Forslind (1966) (later confirmed by Kerr and Drost-Hansen, 1970). French *et al.* speculated on the unusual temperature dependence, and suggested that it "is logically interpreted as a decrease in the effective radius of the scattering centers in this temperature region. The increase in half-life and width represents almost a halving of the effective radius and we can, at present, offer no convincing

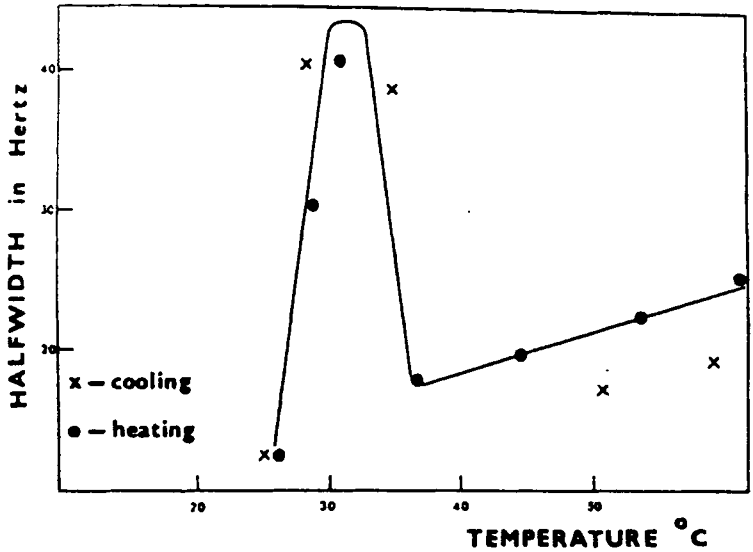

FIGURE 29 Half-width of Raleigh spectrum of concentrated gelatin solution as a function of temperature (scattering angle: 135°; concentration: 70 mg gelatin/ml). (French, Angus & Walton, 1971).

explanation of this phenomenon. In view of the temperature range in which this occurs, we suspect that the collagen segments melt (as in intact collagen) just prior to the disruption of the gel. The precise details are, however, not entirely clear at this stage." In view of the discussion in the present paper, it seems reasonable to suggest that the anomaly is a reflection of the disorganization of the water of hydration in the temperature interval around 30°. Thus, the effective, hydrated radius of the gelatin scattering centers is greatly reduced. An alternative interpretation might be the disruption of vicinal water structuring which may serve to hold together larger numbers of aggregates and/or the decrease in stabilization of collective motion of collagen segments.

Hart, Russell and Cooper (1971) have studied the effects of some glycols and substituted glycols on the thermal stability of soluble collagen. These authors were particularly concerned with the structuring of the vicinal water and proposed that the water structures adjacent to non-polar regions of a collagen molecule (hydrogen-bonded to such a molecule) might be expected to inhibit "the freedom of approach of all other molecules in the vicinity." The authors see this as a mechanism by which the lifetime of a transient, polar bond between solvent molecule and protein might be extended. They observe that "if such water structures have appreciable lifetimes, relative to the frequency of thermal oscillation of the hydrocarbon chain, a considerable buildup of water structure seems feasible."

Over the last few years, Privalov and co-workers have also studied the hydration properties of collagen. Among the techniques used by Privalov and his co-workers is differential thermal analysis. In a particularly interesting study (1970) with Tiktopulo, it was suggested that the thermal denaturation appears to occur in two discrete stages. Thus, the thermogram shows two well developed peaks. However, the optical activity upon denaturation is observed only corresponding to the second stage of the thermal absorption. Thus, the authors conclude that the first step is indeed not a genuine denaturation. Privalov introduced the phrase "sub-denaturation" for these types of changes which mimic the normal denaturation process. The temperature at which these denaturations occur do not necessarily coincide with temperatures for which structural anomalies have been reported by the present author, although some agreement does exist. For instance, the thermal absorption in salt solutions of tropocollagen appears to show an anomaly near 33° (a peak centered around 32½°, beginning at 29.5°). (Privalov, 1969).

Among the interesting studies carried out by Privalov is another study of thermal properties of aqueous procollagen solutions. This study indicated the occasional occurrence of more than two peaks. Privalov and Tiktopoulo (1969) discussed these observations in terms of fusion of morphous regions of the collagen molecule and the possibility of explaining these results in terms of water structuring. Compare, in this connection, the OSB spectroscopic studies by French *et al.* referred to above.

Attention is finally called to the study by Privalov, Tiktopoulo and Serdeyuk (1969) in which the viscosity of highly dilute aqueous solutions of procollagen were measured as a function of temperature at various shear rate gradients. These results clearly showed the occurrence of two stages, respectively around 31–33° and around 34–38°. The specific viscosity behavior of the procollagen solution (0.07%) shows marked increases around 31° for decreasing shear rates. This observation was discussed briefly by the present author (1971).

In connection with thermal properties of proteins, (and the concept of "sub-denaturation") Privalov *et al.* (1971) studied a number of globular proteins and discussed a two stage process of denaturation: a pre-denaturation stage (for which the partial heat capacity changes are tentatively associated with a "labilization of the globular structure") and a "denaturation" as a manifestation of a single step transition. Privalov *et al.* particularly emphasize that it is only for a two stage transition that the van't Hoff equation may be used, assuming that the process is fully reversible. Thus, "the existence of transitions without enthalpy changes makes a simple application of van't Hoff equation suspect. The 'wide popularity' of the use of the van't Hoff equation has simply been based on its simplicity; as is seen now, this simplicity may not exist." Finally, it should be stressed again that the preliminary transitions discussed by Privalov *et al.* may be related to, or a direct manifestation of, the vicinal water structure changes. However, in this connection, it should be noted that the changes in partial heat capacity (for instance of chymotrypsinogen) are strongly dependent upon pH (at least for low pH's) and hence involve more than merely the abrupt change in water structure at only the discrete temperatures discussed in the present paper.

In summary, it appears that many of the observations by Privalov and co-workers may indeed be related to water structure changes and some of these likely identified with the thermal transitions in vicinal water discussed in the present paper. It is felt particularly that "sub-denaturation" may be a manifestation of just the type of conformational changes proposed by the present author (1971) which are imposed by changes in the vicinal water structure.

Ansevin, Spelsburg and co-workers (see Ansevin, 1971 and Spelsburg, 1971) have studied chromatin and artificial nucleohistones by measurements of hyperchromicity as a function of temperature. Of the greatest interest in the present context is the fact that these authors have used their data to calculate the temperature derivatives of the hyperchromicity data. The derivative graphs often reflect the occurrence of multiple peaks, as well as "shoulders" on these peaks. Figure 2 in the article by Ansevin *et al.* shows both the original hyperchromicity curves as well as the temperature derivatives. It is a remarkable example of the degree of resolution attainable and the wealth of additional information which can be obtained by this difference technique. The derivative graphs are interpreted by the authors to reflect both physical as well as chemical features of nucleoprotein complexes. A casual inspection of the results by Ansevin and co-workers (and Spelsburg *et al.*) suggests that abrupt changes in slope are often initiated roughly in the vicinity of 45, 60 and 75°. Recall that thermal anomalies have been observed near 45 and 60°, while the disjoining pressure decreases to zero in the vicinity of 74° (Peschel and Adlfinger, 1971). Recent data (Ling and Drost-Hansen, unpublished) based on DTA measurements of water on Vycor surfaces (see Section V.D, also suggest the occurrence of a possible thermal anomaly in the vicinity of 75°). Thus, it appears likely that the complexity observed by the thermal differentiation technique used by Ansevin, Spelsburg and co-workers, reflect the superimposed changes due to the vicinal water structures.

Finally, other evidence for considerable complexities in the thermal properties of proteins may be seen in the study by Troitsky *et al.* (1971) who studied the reversible conformational transitions of proteins. These "anomalous" changes are frequently observed near 30 to 35°, and around 45° in fair agreement with the temperatures discussed in the present paper for the structural anomalies of vicinal water.

*d Enzymes* Temperature dependent anomalies have been reported in large numbers for aqueous enzyme systems. For enzymes, as for the polypeptides, nucleic acids, and proteins discussed above, conformational changes are expected, a priori, from a knowledge of the complexities of their structures. The main point of the present discussion is to stress that in addition to those conformational changes which are to be expected on purely statistical mechanical grounds for any macromolecule, additional (and in this case more abrupt) changes in properties of aqueous enzyme systems may occur as the result of superposition of the effects of changes in the hydration structures of the enzymes themselves and their substrates. It is stressed again that it is assumed that a *mutual* interaction takes place between the water and the enzyme: in other words, while specific structural details of the enzyme would influence, in part, the local structure of water immediately adjacent to the macromolecule (notably due to ion-dipole, dipole-dipole and similar interactions) it is proposed conversely that the water structure which is expected to be stable over a particular temperature interval, will, in turn, impose conformational constraints on the macromolecules.

Some of the abrupt changes over narrow temperature intervals which have been observed for enzymatic reaction rates (and other properties of enzymes) have been discussed recently by the present author (1971). It is difficult to demonstrate unequivocally that these abrupt thermal changes are due to the vicinal structure. However, three different observations suggest that at least for some enzymes, the higher-order phase transitions of the vicinal water are implicated. These are:

A) changes which are often extremely abrupt, compared to the degree of change observed due to thermal denaturation (for instance, determined from studies of optical dichroism on the purified enzyme).

B) The temperatures at which the thermal anomalies occur are frequently found to be near one of the temperatures where the water in very simple, inorganic systems (such as the quartz/water interface) shows anomalies. Finally,

C) the frequent occurrence of two (or more) abrupt changes.

The latter observation suggests that even if one of the thermal changes (presumably usually the higher temperature change) is indeed due to a conformational change of the enzyme without the specific influence of the water structure, it is somewhat unlikely that *two* such conformational changes should be observed spaced, say, only 15 (to 30)° apart (see discussion of "cold-inactivation" in paper by the present author, 1971).

Over the last few years, Talsky (see 1971) has reviewed some of the examples of anomalous temperature dependences of enzyme catalyzed reactions. Among the conclusions reached by Talsky, one is particularly interesting in connection with the possible role of vicinal water. For the system, trypsin-$N^{\alpha}$-benzoyl-L-arginine ethyl ester, an anomaly is observed at 15.35°C(!)—according to Talsky, "precisely in the anomalous region for the overall reaction." Talsky (personal communication) has not ruled out the possibility that cooperative effects in the vicinal water may be superimposed on the general processes considered. However, while anomalies were found by Talsky near 15, 30 and 45°, anomalies were observed also in other temperature ranges. In this connection, recall however, that the study by Peschel and Adlfinger (1971) suggested that additional thermal anomalies may occur (for instance, near 23°) for water in very close proximity to solid interfaces. (See Figure 4: for distances of 100 Å, several additional peaks are observed in the disjoining pressure). Because the changes observed by Talsky are frequently sensitive to pH, type of substrate, ionic strength, etc., Talsky appears to lean toward an interpretation which does not rely on the anomalous changes in the vicinal water. Talsky has, in his publication, primarily adopted the notion that the anomalies are due to minor, temperature-induced structural changes of the macromolecules. (In this connection, see the discussion in the preceding section of the studies by Privalov where the concept of "sub-denaturation" was introduced). Again, it is stressed here that the phenomenon may still be a reflection merely of the structural transitions in the vicinal water. The articles by Talsky should be carefully studied for the wealth of experimental data presented in these papers.

Lyons and co-workers (see Kumamoto *et al.*, 1971a,b) have also studied anomalous temperature dependencies in Arrhenius graphs for a number of biological systems including some enzymatic rate data. Like the present author, Lyons and co-workers also suggest that the thermal anomalies are manifestations of phase changes. The main difference between Lyons and co-workers on the one hand and the present author on the other hand, seems to be in the material to which such phase changes are ascribed. Obviously, the present author prefers to interpret the anomalies in terms of phase transitions in the vicinal water, while it appears that Lyons and co-workers—like Talsky—appear to favor conformational changes of the macromolecules. Perhaps the most remarkable results obtained by Lyons and co-workers are the case where actual, overt discontinuities are suggested in the Arrhenius plots. However, as best as one can tell from the published data, only one "solid" example appears to have been produced for such an overt discontinuity—namely for the succinate oxidation by mitochondria from cucumber. In this case, the anomally appears centered around 12°; for the succinate oxidation by rat liver mitochondria, the anomaly appears to occur at approximately 23°. Note, incidentally, that for the same process utilizing enzymes from the mitochondria of sweet potato roots, or tomato fruits, the anomaly appears centered around 10 to 11°.

For an interesting example of a temperature anomally in enzyme kinetics, see the article by Sipos and Merkel (1970). These authors have studied the heat stability and susceptibility to calcium ion of the structure of trypsin. Anomalies in the activity of the enzyme were obtained in the form of sharp maxima near 45 and approximately 62°.

### C Current state of NMR studies of biological water

Nuclear magnetic resonance and particularly proton magnetic resonance appear promising as powerful tools for elucidating the structure of water in biological systems. Indeed, a large number of studies have been made, both on living biological systems and in vitro, on such model systems as gels and solutions of macromolecular solutes. Notwithstanding the impressive number of studies which have been carried out, particularly since 1957, relatively little has been proven with any degree of certainty by this experimental approach. In fact, the conclusions to date appear controversial. It does seem as if the majority of investigators studying living systems have concluded that at least some water in biological systems (especially in muscle) is structurally different from the bulk. Various authors have suggested the existence of more than one fraction of oriented water. Unfortunately, a number of experimental problems continue to detract from some of these conclusions, such as the possible effects of paramagnetic impurities, sample inhomogeneities, etc. An excellent survey of the current state of the art was presented at the New York Academy of Sciences "International Conference on Physico-Chemical State of Ions and Water in Living Tissues and Model Systems" (Hazlewood, editor, 1972). For a well annotated, condensed survey of nuclear magnetic resonance investigations of cell water, see Walter and Hope (1971).

A brief but eloquent defense of the validity of NMR studies of water in biological systems has come from Chang *et al.* (1972). These authors reviewed the notion of ordered water in biological tissues and ruled out various criticisms of this interpretation. Chang *et al.* further suggested that "the change of water properties (in the NMR sense) in the cellular environment is a universal phenomenon which is independent of species and tissue." This statement is indeed much akin to the "paradoxical effect" discussed in Section IV.C.1. The authors also argue against the suggestion by Glasel (1968) that no ordering of water need be postulated, based on his NMR experiments on glass beads in water. In fact, Chang *et al.* conclude that Glasel's experiments support rather than disprove the contention of the existence of ordered water near interfaces.

For a representative example of the "dissident opinions" regarding nuclear magnetic resonance, evidence for water structuring effects near interfaces, see for instance, the paper by Hansen (1971). Using both pulsed and wide-line NMR, Hansen studied $D_2O$ in rat muscle and brain tissue, and interpreted the results in terms of the self-diffusion of the water, similar to that of bulk water except for the "tortuosity" of the water, encountering physical barriers. As a result, Hansen concluded that no evidence exists for a fraction of water with greatly restricted molecular mobility. The reason for the divergent results obtained by Hansen compared to the conclusions of most other current studies remains a puzzle. For further discussion, see the Annals (Hazlewood, editor) of the New York Academy of Sciences Meeting, January 1972).

## D Entropy-enthalpy compensation phenomenon

### *1 Study by Lumry and Rajender*

Lumry and Rajender (1970) have recently discussed the entropy-enthalpy compensation phenomenon, particularly with respect to biological systems. Characteristically, it is possible to fit experimental data to an equation of the type:

$$\Delta S^* = a\Delta H^* + b$$

In this equation, the constant $a$ has the dimension of reciprocal absolute temperature; this temperature, $T_c$, is often referred to as the compensation temperature. For many of the systems examined by Lumry and Rajender, the characteristic compensation temperature falls in the range from 285 to 290°K. The implications of this were discussed by these authors and more recently by the present author (1971). It is interesting to note that this temperature range is centered around the range of the temperature for the lower transition in vicinal water, namely 15°C (288°K).

In connection with the entropy–enthalpy compensation phenomena, see the article by Lovrien (1969), who also called attention to the fact that the compensation temperature is often approximately 280°K (±about 20°K) (when water is the solvent—in contrast to vastly different values for organic solvents). Lovrien concluded "$\Delta H^*$ and $\Delta S^*$ are coupled by means of macromolecular structure, or more properly, by means of reactions intimately involving the dynamics of macromolecular structure. In turn, these are controlled, partly or largely, by the structure or other properties of water."

### 2 *Thermal death mechanism*

The mechanism of thermal death remains obscure; a review of this topic can be found in a paper by Fry (1967) and a brief review was presented by the present author (1971). Rosenberg and co-workers (1971) have studied the entropy–enthalpy compensation phenomena for denaturation of proteins and compared the values with those obtained for the thermal death of a number of organisms. Their results are summarized in Table V below. In this connection, attention is called to the narrow range of the characteristic compensation temperatures observed. For the four systems studied, the average temperature is 56°C; hence, it is tempting to speculate that this temperature may be related to the temperature range for the (upper) thermal anomaly for vicinal water, discussed by the present author, namely the range from approximately 59 to 62°C. [Recall, Pasteurization temperature is 60–62°C; see Drost-Hansen, 1965].

For a review of earlier studies and an introduction to the entropy–enthalpy compensation phenomenon, see the paper by Likhtenshtein (1966). One of the most interesting conclusions reached by Likhtenshtein is the suggestion that "the enzymatic process must be accompanied by periodic changes in configurational structure of a certain part of the protein or *its hydration shell* with a frequency approximately corresponding to the turnover number of the enzyme" (emphasis by present author). In a sense, then, this suggests the same relationship as stressed independently by the present author (1971) regarding the *mutual* interaction between macromolecular conformation and stabilities of vicinal water structures.

Glick (1971) has determined the temperature dependence of hydrolysis by chemotrypsin. One of Glick's conclusions is particularly interesting. He suggests that the "near coincidence" of the critical temperature (the proportionality factor in the entropy–enthalpy plot) and the temperature at which the Arrhenius plot shows a change in slope suggests that thermally induced conformational changes may be the underlying cause of the experimental manifestations. Specifically, Glick suggests that the transition state is related

TABLE V

Comparison of compensation law constants, $T_c$ and $b$, from: $\Delta S^{\ddagger} = a\Delta H^{\ddagger} + b$; $T_c = 1/a$

| | $T_c$( K) | $T_c$(°C) | $b$(calories/mol × K) |
|---|---|---|---|
| Proteins | 329 | 56 | −64.9 |
| Virus | 330 | 57 | −64.0 |
| Yeasts | 325 | 52 | −64.5 |
| Bacteria | 331 | 58 | −65.0 |

to that of the complex by the same increase in $\Delta F$ so as to allow "water structure to intervene."

## E Various studies by other authors

Over the past few years; the state of water in biological systems has begun to attract increasing attention. Because of space limitations, it is not possible here to do justice to all or even some of the authors who have contributed significantly to this field. Instead, reference is made to the recent survey by the present author (1971) and attention is called specifically to a number of authors who have contributed significant papers: these include Lewin (1970; 1971a,b); Walter and Hope (1971a,b); Cope (1969; 1971); Ling (1962; 1965); Damadien (1971a,b,c,d); Sõremark (1972) and Robinson (1970). Only the results by one author will be elaborated upon as an example of careful studies by rather traditional means.

### *1 Study by Hinke*

Hinke (1970) has studied the water in the muscle fiber of the giant barnacle, *Balanus nubilus*. Seven different types of experiments were described, each permitting an estimate of that water which acts as "solvent water" or is "osmotically active." Four sets of experiments were made, using ion selective microelectrodes, namely for sodium, potassium and chloride ions. One experiment made use of a membrane potential vs. known potassium ion activity, while the last two experiments determined the water concentration via an osmotic phenomenon determining fiber volume measuring its buoyant weight in two medias of known densities. The results are impressive in that for all seven independent estimates, the amount of "essentially normal solvent water" ranged from 0.64 to 0.72 of the water contained in the fiber. The average was 0.68. The extracellular space was estimated to be about 7%, which when added to the 68% leaves a total of 25% "analyzable water, not acting as solvent." In a sense, the experiments by Hinke are an "indirect way" of getting after the topic of interest, namely the differently structured water: this is undoubtedly the water which, in the present paper, is referred to as the vicinal water of the system. Possible objections can be raised to this approach by implicating ion activity changes due to some type of complexing between, for instance, the cations (primarily sodium and potassium) with the macromolecular solutes of the intracellular protoplasm. However, in connection with the nuclear magnetic resonance studies, the dielectric studies, and the studies of thermal anomalies stressed by the present author, it appears that the evidence is certainly substantial in favor of an interpretation in terms of differing ion activities due directly to water structuring effects.

## F Active transport

The question of the nature and degree of structuring of intracellular water may be of crucial importance to our understanding of active transport. In the past it has been difficult to demonstrate unequivocally the reality of active transport primarily because of a lack of suitable "trans-membrane carriers." The materials which have been alleged to be responsible for the transport against stoichiometric concentration gradients have most frequently been postulated to be proteins (referred to as transport proteins, permeases, translocases, transfer locases, etc.). The most embarrassing problem has been the need for postulating essentially a different transport carrier for each ion moving across the membrane (against a concentration gradient). A second difficulty has been in the mechanism of coupling to metabolic energy to effect the transmembrane transport.

The alternative to active transport is the notion that while indeed stoichiometric concentration gradients exist and ions move against these gradients, the movement may actually be in the direction determined by changes in the ion activities. Thus, the ions in the intracellular water may be either

A) somehow complexed by functional groups (mostly ionic sites) on the macromolecules in the interior of the cell, or

B) the ion activities may be changed due merely to the different structuring of the intracellular water (or both of the phenomena (A) and (B) may be operating.

In either case, the ion activities are notably changed in the interior of the cell. Hence, in the interpretation in terms of enhanced water structuring—leading to a reduction in ion activity—both of the major problems regarding active transport theory are overcome to some extent: no specific, different trans-membrane carrier is required for each ion moving across a membrane and it becomes somewhat easier (but by no means simple) to explain the "coupling" to metabolic energy. "Discrimination" between various ions is proposed to be the result of the different types of interactions possible between the hydration shells of the ions and the different water structures present in the vicinal water. The energy expenditure is essentially only that required to maintain the structured nature of the intracellular water—once the structure has become established. For a recent study of these possibilities, see the article by Wiggins (1971); see also a "classical approach" in the paper by DeHaven and Shaprio (1968).

The likely effects of a surface on the vicinal liquid have been stressed in the past by several authors, but few have done so more eloquently than Ubbelohde.

At the Faraday Symposium on Membranes (1956), Ubbelohde observed that "the quasi-crystalline structure of liquids such as water, ethyl alcohol, may undergo profound changes near a surface, especially when the surface contains polar molecules as is the case for many membrane walls." Ubbelohde goes on to note that several properties should be significantly affected, including

I) the effective viscosity—leading to different entropies and energies of activation for viscous flow;

II) ion mobilities differing from the values in bulk solution; and finally

III) ion activities which may be appreciably changed, both in the "diffusion layer" (nearest to the walls) as well as in the "channels," i.e. in the lumen of relatively narrow pores.

Ubbelohde, incidentally, also called attention to the heat transfer which might accompany the flow of liquid through the membrane due to the consecutive transitions from liquid with bulk structure (I) → modified layers → to bulk fluid (II). With these remarks, the stage was actually set for the models for active transport which are now developing (see, for instance, Wiggins, 1971, or Dragomir, 1971).

In Section V.A, and Section IV.B.5, the possible consequences for the dielectric properties of water were discussed in terms of vicinal water structuring. Unfortunately, at present our understanding of the dielectric properties of water near interfaces in general, and near biological interfaces in particular, is in an unsatisfactory, qualitative state. However, if advances can be made along these lines, it appears possible that semi-quantitative theories for ion transport might be developed by some extension of classical electrolyte solution theory (such as extension of the Debye-Hückel theory, using the "correct" values for the dielectric constants of the intracellular water for calculations of ion activities). The paper by DeHaven and Shapiro (1968) might be a good starting point in this connection.

An interesting model for active transport across cell membranes has been proposed by Dragomir (1971). In this treatment, an attempt is made to model the trans-membrane transport by a process of "zone melting." The essence of the model is the passage through a pore in a membrane of a plug of differently structured phase of water. A "distribution coefficient" for ions, strongly differing from one, is assumed between the structured water of the cell (and cell pore) and the unstructured "plug" of water moving through the pore; the model thus invokes a phase transition (and assumes that pores exist in membranes). The suggestion is made that "the pump activity takes place as a result of some revolving, continuous and traversally limited changes of the polarization–depolarization type." Ordinary Ice-I-like water, is not necessarily invoked, merely differently structured water. However, difficulties appear in explaining the mechanism of the driving energy for the process.

Dragomir claims that ATP-lysis supplies the required energy but the mechanism by which "this compound, falling off at the inner end of the pore, is supposed to bring about protein structure modifications which propagate along the pore wall, giving rise to the phase transition" is far from clear. The present author does not subscribe to the details of the mechanism proposed by Dragomir. However, the fundamental idea of considering structuring in cells, and/or in membrane pores as the cause of redistribution of ions is indeed a tempting one. Although somewhat misguided, Workman and the present author (1954) suggested essentially a similar mechanism for the generation of transient potentials in biological systems, particularly nerves. (A more recent attempt at a quantitative theory of ion distribution in living tissue, based on the interaction between structured water and the hydration structures of various ions, was given by Wiggins, 1971). Before concluding this section, attention is again called to the fact that while "high energy" interactions (of the type ion–ion and ion–dipole) must certainly play a notable role in determining ion distributions in cellular systems, the weak interactions between the solvent molecules themselves must not be overlooked. In fact, as stressed in the present paper, it is possibly this type of weak interactions which are responsible primarily or exclusively for many cooperative phenomena in cellular systems (in addition, of course, to those determined by conformational changes of the macromolecules and lipid phase transformations).

## VIII NON-EQUILIBRIUM ASPECTS OF BIOLOGICAL SYSTEMS

### A Dissipative processes as a source of time and space ordering

Recently, Glansdorff and Prigogine (1971) have studied systems under conditions far from equilibrium. The study is remarkable in demonstrating how systems very far from an equilibrium state may become ordered into extensive (local) domains as a means of approaching equilibrium via the dissipative processes. The domains of ordering may range from macroscopic dimensions for molecular systems to megascopic (such as in the Benard problem of major convective currents in the ocean). In connection with the study by Glansdorff and Prigogine, it is interesting to speculate that ordering (and cooperativity) of water in biological systems plays a decisive role in the difference between the "quick and the dead."

As discussed by Glansdorff and Prigogine, systems very far removed from equilibrium may achieve stability through partitioning into domains of local order. It is proposed here that the ordering of the vicinal water structure may perhaps be thought of as one such subset of order within the entire system. It is obvious that the macromolecules (such as the protein and the lipids) may form ordered structures and that this attribute is essential to biological functioning. But it is now tempting and perhaps necessary—to add to these "subsets" of partial ordering—the specific subset consisting of vicinal, structured water. By reason of sheer abundance, the water may play the dominant role. It is indeed well cast for this role by virtue of the existence of weak interactions. Thus, the functioning of the biological systems may be seen as the maintenance of local order under decidedly non-equilibrium circumstances, but requiring presumably only a minimum amount of metabolic energy (as compared to such processes as "active transport" requiring "stoichiometric amounts" of energy). The mechanism by which metabolic energy—in one form or another—effects the necessary ordering of the vicinal water is obscure at this time. However, it should be kept in mind, of course, that some type of vicinal ordering also occurs as an equilibrium phenomenon (requiring no expenditure of energy) near a variety of interfaces, completely independent of any attributes of life. The converse, however, is possibly not true. Thus, thermal death may well represent the local destruction of one of the required subsets of order—present only because of the system being in a state far from equilibrium. The disordering may allow for distinctly different rates of approach to equilibrium. Equilibrium is death.

Glansdorff and Prigogine also note that "when such instabilities occur, entirely new properties arise. The structure and properties of the solution below the instability point cannot be extrapolated, even in a first approximation. Still, we remain in the range where a macroscopic description should be possible. Indeed, all these phenomena may occur in dense media, where the number of collisions is sufficient to maintain the equilibrium conditions on the *macroscopic level*." In a discussion of membrane excitability, Glansdorff and Prigogine attempt to "show how cooperativity is affected by the nonequilibrium constraints. The results reported here certainly indicate how the cooperative *molecular* properties of membrane units can be amplified by energy dissipation and account for the phenomenon of membrane excitability." Thus, this leads Glansdorff and Prigogine to note that "the state at rest of a membrane corresponds to a molecular organization which is separated from its excited state by a discontinuity; the jump across this discontinuity, following small perturbations, constitutes the membrane excitation process. The excited state itself is obtained by gradual deviation from equilibrium. The excitation process is based on the coupling of a cooperative structural transition of the membrane, with a downhill translocation of ions. We may therefore qualify membrane excitation as an *assisted phase transition*, as it appears in our model." The present author suggests that one of the possible and indeed likely elements to exhibit ordering and cooperative properties is the vicinal water.

## B Dipolar aspects and non-equilibration of thermal energy

Somewhat similar to the approach by Prigogine (but independently) Fröhlich (1968) has considered cooperative effects in biological systems. Fröhlich begins by pointing out that many important aspects of the physics of materials can be described in terms of the collective (i.e., cooperative) behavior "of a great number of these constituents only, usually associated with some kind of long-range order. Most frequently, this order is described in terms of a definite spatial pattern." However, Fröhlich goes on to point out that a far more subtle type of long-range order is found in such materials as superfluid helium and in super-conductors. In these cases, the order is associated with long-range phase correlation (coherence). Prompted by the extraordinary dilectric properties of biological systems, it occurred to Fröhlich that some unique result might derive from longitudinal electrical modes of vibration in these systems. He begins his analysis with three general statements about the physical living systems. These are

a) the relative stability in spite of being far from thermal equilibrium;

b) the suggestion that some type of order may exist within them, but without being obviously spatial in nature; and

c) that cells (and many of the macromolecules of cells) have unique dielectric properties.

On this basis, Fröhlich proceeds to consider a suspension of units capable of dipole oscillations. These oscillations, via long-range Coulomb interactions, then derive to a branch of longitudinal electric modes within a relatively narrow frequency domain. As "relevant components" in a biological system, Fröhlich suggests parts of the cell membrane with certain chemical bonds "particularly with hydrogen bonds" or with regions containing non-localized electrons. From the theoretical discussion, Fröhlich concludes that if the rate of energy supplied is sufficiently large, this energy will be channeled into a single mode "which then presents a strongly excited, coherent, longitudinal electric vibration. Its wave length depends on the details of the geometric arrangement of the components. A certain fraction of this applied energy, therefore, is not thermalized, but stored in a single mode, which then exhibits long-range phase correlation. This, then, may lead to the maintenance through the energy flux of a state of high order far from thermal equilibrium." In connection with these considerations, it is obvious that the water associated with the macromolecules may play an important role and possibly particularly the water structure entities discussed in this paper (as opposed to the "tightly bonded" waters of interaction with dipolar or ionic sites on the macromolecular solutes). Possibly, the state of "enhanced order" is an alternate way of describing the energy delocalization effect. Note, of course, that we must carefully make the distinction between such organized water elements in living systems as distinct from the definitely thermally equilibrated, stable, enhanced structures of vicinal water adjacent to many, non-living, inorganic solid surfaces.

A somewhat different and slightly more quantitative approach has been attempted by Lavenda (1971) who has considered cooperative effects derived from charge fluctuation in some specific physical systems. Already twenty years ago, Kirkwood and Schumaker (1952) drew attention to the fact that charge fluctuation forces may account for a substantial contribution to the thermodynamics of protein molecules in solution. In order to achieve such effects, the forces must be long-range and non-specific. Coulombic interactions constitute such a type of force. These forces will become particularly prominent in media of low dielectric constants. From his computations, Lavenda concludes that the fluctuation forces may, under conditions of non-equilibrium, amplify the effective range of the charge fluctuation forces to the point where they may become important in determining the macroscopic order of the system.

### C "Aperiodic crystals" and exclusion of disorder in cellular systems

Mendelssohn (1969) has, in a very popular way, speculated on the role of ordering as related to the physical basis of life. Mendelssohn points out that the current trend in molecular biology is the explanation of life as the "geometric fitting of chemical bonds between individual atoms." The order pattern involved would be one according to positions or, as Schrödinger once called it, an "aperiodic crystal." Mendelssohn has also argued that in the space which is occupied by life "randomness is always excluded to a remarkable extent." This again, may perhaps be an alternate way of expressing the ideas of Glansdorff and Prigogine.

In view of this notion, Mendelssohn concludes that possibly the mere local ordering, as in an aperiodic crystal, may lead to the result that the "limitation of the position order patterns lies in the short range of information that reaches essentially no further than the nearest atomic neighbor. This distance seems far too short to account for the large-scale aperiodic pattern required for excluding randomness." It is in this connection that large-scale, extensive cooperative interactions among the water molecules of the cell may serve to bridge the gap between crystalline attributes and the aperiodic crystals.

Finally, Mendelssohn points out that most descriptions of order patterns are based on *either* position *or* momentum and suggests that one may be forced to consider "mixed condensates employing both types of order." In summary, Mendelssohn concludes "one can expect these aggregates to have a distinct shape, and at the same time to exhibit a coherence that goes far beyond the interatomic distance. In fact, they would look suspiciously like living matter."

In connection with the considerations of the overall energetics of cellular organisms, it is also of interest to note the remarks made by Forrest (1970), who studied the entropy production associated with the growth of various anaerobic organisms (utilizing glucose as the energy source). From the results of four different organisms, Forrest concludes that apparently only a small fraction of the available energy is incorporated into cellular material. Instead, "the major thermodynamic process is a large outflow of entropy from the growing cells. There is evidence for efficient energetic coupling during growth (Ref. 23), so that the energy is not wasted. It follows that the major consumption of energy is that required for the correct placement of molecules within the cells into organized groups or structures." In the present context, it is tempting to associate this entropy flow with the ordering of the water molecules in the cells. Recall again that, on the average, 70% of the cell mass is water and recall the low molecular weight: hence, the relatively large number of moles of water involved per unit mass of cell.

## IX SUMMARY AND CONCLUSIONS

Four questions have been posed in this paper, namely:

A) does the structure of vicinal water differ significantly from the structure of bulk water?

B) What structured entities (if any) predominate in vicinal water?

C) Over what distances do such structures extend, and finally

D) What properties does the vicinal water possess?

In addition to these questions, some comments have been made in light of the answers to the four basic questions regarding the structural and functional role of water at biological interfaces.

It appears fairly certain that the properties of vicinal water are distinctly different from the properties of bulk water. In some cases, the differences are very pronounced (for instance, heat conductivity, vapor pressure, dielectric properties, disjoining pressure and surface entropy). Such notable changes in properties must reflect structural differences. Unfortunately, "the crystallographic" nature (if it is permissible to speak about such) of the vicinal water remains obscure. Possible candidates for the vicinal structures are clathrate hydrates and high pressure ice polymorphs; the existence of other structures, however, must be considered possible. No firm estimates can be given for the distances over which the modified vicinal structures may extend. In the opinion of the present author, a "minimum guess" would be at least around 0.01 microns and a more likely estimate would be of the order of 0.1 microns. It is important to recognize that these structures exhibit cooperative hydrogen bonding with energies only slightly different from bulk water. The evidence for the existence of thermal transitions (likely higher-order phase transitions) appears clearly established.

In connection with biological systems, the most important aspect is undoubtedly the distance over which the vicinal structures extend—being comparable to intracellular distances. The second most important attribute is the ability to undergo (higher-order) phase transitions as these appear to profoundly influence many macromolecular and cellular systems. Effects are likely "sensed" by cellular systems via the abrupt changes in nature and extent of the hydration of macromolecules, and/or vicinal structures in and adjacent to membranes. The "paradoxical effect" is the non-specific influence of the type of surfaces to which the water is adjacent—apparently relatively insensitive to whether the surface is hydrophobic or hydrophilic (apart from the specific, "high-energy" hydration phenomena associated with ionic and strongly dipolar sites). Finally, it is proposed that the ability of water to exhibit very long-range, low energy structuring may be an important element in seeking means for achieving extended domains of structuring in dissipative processes under conditions far from equilibrium, such as encountered in living cells.

## Acknowledgements

The author wishes to acknowledge the support by the Office of Saline Water for his research on the structure and properties of water, particularly water in membrane systems and at the ice/water interface. The author also gratefully acknowledges many discussions, criticisms, and comments from Drs. R. Benach, G. A. Clarke, J. S. Clegg, and C. R. Hare, and the helpful assistance with the editing by Miss Christine E. Drost-Hansen.

## References

1. F. F. Abraham and J. V. Dave, *Chem. Phys. Let.*, **8**, 351 (1971).
2. N. V. Afanas'ev, M. S. Metsik, and V. N. Popova, *Research in Surface Forces*, **2**, B. V. Derjaguin, Ed., p. 176, Plenum Press, Consultants Bureau Publishers, 1966.
3. A. T. Ansevin, L. S. Hnilica, T. C. Spelsberg, and S. L. Kehm, *Biochemistry*, **10**, 4793 (1971).
4. K. Arakawa and K. Sasaki, *Bull., Chem. Soc. Japan*, **43**, 3048 (1970).
5. E. M. Arnett, M. Ho, and L. L. Schaleger, *J. Amer. Chem. Soc.*, **92**, 7039 (1970).
6. N. G. Bakradze, D. R. Monaselidze, G. M. Mrevlishvili, A. D. Bibikova, and L. L. Kisselev, *Biochim Biophys. Acta*, **238**, 161 (1971).
7. C. Ballario, A. Bonincontro, C. Cametti, and S. D'Agostino, *Nature, Physical Science*, **234**, 179 (1971).

8. H. J. C. Berendsen, *Fed. Proc.*, **25**, 971 (1966).
9. H. J. C. Berendsen and C. Migchelsen, Proceedings of Seminar in Biophysics and Physical Chemistry of Connective Tissue, *Fed. Proc.*, **998** (1966).
10. H. J. C. Berendsen, *Theoretical and Experimental Biophysics*, A. Cole, Ed., p. 1, Marcel Dekker Publisher, 1967.
11. J. D. Bernal and R. H. Fowler, *J. Chem. Phys.*, **1**, 515 (1933).
12. J. D. Bernal, *Nature*, **185**, 68 (1960).
13. M. Blank and V. K. LaMer, *Gas/Liquid and Liquid/Liquid Interface*, Proceedings of 2nd International Congress of Surface Activity, Butterworths Scientific Publishers, London, 1957.
14. M. Blank and V. K. LaMer, *J. Coll. Inter. Sci.*, **13**, 102 (1958).
15. A. J. H. Boerboom and G. Kleyn, *J. Chem. Phys.*, **50**, 1086 (1969).
16. J. J. Burton, *Chem. Phys. Let.*, **7**, 567 (1970).
17. J. J. Burton, *Nature*, **229**, 335 (1971).
18. R. E. Carpenter and J. B. Graham, *Comp. Biochem. Physiol.*, **22**, 709 (1967).
19. R. C. Chandan, J. Cullen, B. D. Ladbrooke, and D. Chapman, *J. Dairy Science*, **54**, 1744 (1971).
20. D. C. Chang, C. F. Hazlewood, B. L. Nichols, and H. E. Rorschach, *Nature*, **235**, 170 (1972).
21. D. Chapman and D. F. H. Wallach, *Biological Membranes—Physical Fact and Function*, p. 125, Academic Press, Inc., 1968.
22. D. Chapman, *Biological Membranes—Physical Fact and Function*, Academic Press, N.Y., 1968, 438 pp.
23. J. S. Chaudhry and R. K. Mishra, *Studia Biophys.*, **17**, 99 (1969).
24. R. Cini, G. Loglio, and A. Ficalbi, *Nature*, **223**, 1148 (1969).
25. R. Cini, G. Loglio, and A. Ficalbi, *J. Coll. Inter. Sci.*, 1972, in press.
26. J. Clifford, J. Oakes, and G. J. T. Tiddy, *Disc. Faraday Soc.*, Thin Liquid Films and Boundary Layers, 175 (1970).
27. K. S. Cole, *Membranes, Ions and Impulses. A Chapter of Classical Biophysics*, Univ. of Calif. Press, Berkeley, 1968, 569 pp.
28. F. W. Cope, *Biophys. J.*, **9**, 303 (1969).
29. F. W. Cope, Symposium on Water Structure at the Water-Polymer Interface, American Chemical Society, 1971.
30. H. G. L. Coster and R. Simons, *Biochim. Biophys. Acta*, **203**, 17 (1970).
31. A. Cownie and L. S. Palmer, *Proc. Royal Soc.* (London), Series B, **65**, 295 (1952).
32. R. Damadien, *Biophys. J.*, **11**, 739 (1971a).
33. R. Damadien, *Biophys. J.*, **11**, 761 (1971b).
34. R. Damadien, *Biophys. J.*, **11**, 773 (1971c).
35. G. Dantl and I. Gregora, *Naturw.*, **55**, 176 (1968).
36. J. V. Dave and F. F. Abraham, *Surface Science*, **26**, 557 (1971).
37. R. J. H. Davies and N. Davidson, *Biopolymers*, **10**, 21 (1971a).
38. R. J. H. Davies and N. Davidson, *Biopolymers*, **10**, 1455 (1971b).
39. W. W. Dean and J. Lebowitz, *Nature, New Biology*, **231**, 5 (1971).
40. J. C. DeHaven and N. Z. Shapiro, *Persp. Biol. Med.*, **12**, 31 (1968).
41. R. E. Dehl, *J. Chem. Phys.*, **48**, 831 (1968).
42. J. E. Del Bene and J. A. Pople, *Chem. Phys. Let.*, **4**, 426 (1969).
43. J. E. Del Bene, *J. Chem. Phys.*, **55**, 4633 (1971).
44. B. V. Derjaguin, *Research in Surface Forces*, **2**, Plenum Press Consultants Bureau, 1966, 320 pp.
45. J. N. Dickey and A. Paskin, *Phys. Rev.* **1B**, 851 (1970).
46. C. T. Dragomir, *J. Theor. Biol.*, **31**, 453 (1971).
47. G. Dreyer, E. Kahrig, D. Kirstein, J. Erpenbeck, and F. Lange, *Zeit. f. Naturf.*, **23a**, 498 (1968).
48. G. Dreyer, E. Kahrig, D. Kirstein, J. Erpenbeck, and F. Lange, *Naturw.*, **56**, 558 (1969).
49. W. Drost-Hansen, *N.Y. Acad. Sci. Annals*, **125**, 471 (1965a).
50. W. Drost-Hansen, *Ind. Engr. Chem.*, **57**, (3-4), 39 (1965b).

51. W. Drost-Hansen, Proceedings, First International Symposium on Water Desalination, October (1965), Vol. 1, 382; U.S. Gov't. Printing Office, 1967.
52. W. Drost-Hansen, Second International Biophysics Congress, Vienna, Austria, 1966.
53. W. Drost-Hansen and A. Thorhaug, *Nature*, **215**, 506 (1967a).
54. W. Drost-Hansen, *Advances in Chemistry Series*, **67**, 70 (1967b).
55. W. Drost-Hansen, *J. Coll. Inter. Sci.*, **25**, 131 (1967c).
56. W. Drost-Hansen, *J. Phys. Chem.*, **73**, 34 (1969a).
57. W. Drost-Hansen, *Chem. Phys. Let.*, **2**, 647 (1969b).
58. W. Drost-Hansen, *Ind. Engr. Chem.*, **61**, 10 (1969c).
59. W. Drost-Hansen, *Chesapeake Sci.*, **10**, 281 (1969d).
60. W. Drost-Hansen, *Physical Principles of Biological Membranes*, Snell *et al.*, Ed., p. 243, Gordon & Breach Sci. Publishers, 1970.
61. W. Drost-Hansen, *Chemistry of the Cell Interface*, **B**, H. D. Brown, Ed., p. 1, Academic Press, 1971.
62. W. Drost-Hansen, *Fed. Proc.*, **30**, 1539 (1971).
63. W. Drost-Hansen, *SEB Proceedings*, XXVI, p. 61–101, 1972a.
64. W. Drost-Hansen, *J. Geophys. Res.*, 77 (27), p. 5132–5146, 1972b.
65. W. Drost-Hansen, *Annals, N.Y. Acad. Sci.*, Vol. 204, p. 100–112, 1973.
66. G. Ebert, *Kolloid Zeit. Zeit. f. Polymere*, **198**, 65 (1964).
67. D. Eisenberg and W. Kauzmann, The Structure and Properties of Water, Oxford University Press, N.Y., 1969, 296 pp.
68. R. P. Enever and N. Pilpel, *Trans. Far. Soc.*, **63**, 781 (1967).
69. W. G. Flamm, M. McCallum, and P. M. B. Walker, *Proc. Nat. Acad. Sci.*, **57**, 1729 (1967).
70. N. Fletcher, *The Chemical Physics of Ice*, Cambridge University Press, Cambridge, 1970, 271 pp.
71. W. W. Forrest, *Nature*, **225**, 1165 (1970).
72. E. Forslind, *Svensk. Naturv.*, **2**, 9 (1966).
73. F. C. Frank, *Acta Cryst.*, **4**, 497 (1951).
74. H. S. Frank and W. Y. Wen, *Disc. Far. Soc.*, **24**, 133 (1957).
75. H. S. Frank, *Proc. Roy. Soc.*, **A247**, 481 (1958).
76. H. S. Frank and A. S. Quist, *J. Chem. Phys.*, **34**, 604 (1961).
77. H. S. Frank, *Science*, **169**, 635 (1970).
78. F. Franks and D. J. G. Ives, *J. Chem. Soc.*, 741 (1960).
79. F. Franks, J. Ravenhill, P. A. Egelstaff, and D. I. Page, Research Report, Colworth/Welwyn Laboratory, 35 (1969).
80. M. J. French, J. C. Angus, and A. G. Walton, *Biochim. Biophys. Acta*, **251**, 320 (1971).
81. Harold Friedman (personal communication, 1972).
82. H. Fröhlich, *Int. J. Quan. Chem.*, **11**, 641 (1968).
83. V. P. Frontas'ev, *Dokl. Akad. Nauk. SSSR*, **111**, 1014 (1956).
84. F. E. J. Fry, *Thermobiology*, Academic Press, N.Y., 375 (1967).
85. G. Gawalek, *Einschlussverbindungen, Additionsverbindungen, Clathrate*, Deut. Verlag Wiss., Berlin, 1969.
86. T. H. Gawronski and F. Wold, *Biochemistry*, **11**, 440 (1972).
87. G. J. Gittens, *J. Coll. Inter. Sci.*, **30**, 406 (1969).
88. P. Glansdorff and I. Prigogine, *Thermodynamic Theory of Structure, Stability and Fluctuation*, Wiley/Interscience Publishers, London, 1971, 306 pp.
89. J. A. Glasel, *Nature*, **218**, 953 (1968).
90. J. A. Glasel, *J. Am. Chem. Soc.*, **92**, 372 (1970a).
91. J. A. Glasel, *J. Am. Chem. Soc.*, **92**, 375 (1970b).
92. D. N. Glew, *Nature*, **195**, 698 (1962).
93. D. M. Glick, *Biochim. Biophys. Acta*, **250**, 390 (1971).
94. B. J. Goldsmith and J. Muir, *Trans. Far. Soc.*, **56**, 1656 (1960).
95. J. Graham, G. F. Walker, and G. W. West, *J. Chem. Phys.*, **40**, 540 (1964).
96. R. C. Grant, M. Kodama, and R. D. Wells, *Biochemistry*, **11**, 805 (1972).
97. G. W. Gross, Final Report ONR, Dept. of Navy, Contr. No. 815, 1970.
98. J. R. Hansen, *Biochim. Biophys. Acta*, **230**, 482 (1971).

99. G. J. Hart, A. E. Russell, and D. R. Cooper, *Biochem. J.*, **125**, 599 (1971).
100. L. V. Heilbrunn, *Am. J. Physiol.*, **68**, 645 (1924).
101. J. C. Henniker, *Rev. Mod. Phys.*, **21**, 322 (1949).
102. S. A. Henry and A. D. Keith, *Chem. Phys. Lipids*, **7**, 245 (1971).
103. D. K. Heydinger, E. J. Hammer, R. W. Pfeil, and P. H. Taylor, *J. Lab. Clin. Med.*, March (1971).
104. K. Hickman and I. White, *Science*, **172**, 718 (1971).
105. J. Hijmans, Phase Transitions, Proceedings, XIV Solvay Conference, Brussels, p. 241, 1969.
106. J. H. Hildebrand, *Proc. Nat. Acad. Sci.*, **64**, 1329 (1969).
107. J. A. M. Hinke, *J. Gen. Phys.*, **56**, 521 (1970).
108. T. Hori, U.S. Army Snow, Ice and Permafrost Research Estab., Translation No. 62, 1960, 8 pp.
109. R. A. Horne, Water in Aqueous Solutions: Structure, Thermodynamics, and Transport Processes, Wiley-Interscience, N.Y., 1972.
110. A. Ilani, *Biochim. Biophys. Acta*, **94**, 405 (1965).
111. B. Jacobson, *Nature*, **172**, 666 (1953).
112. B. Jacobson, *Svensk. Kem. Tidskr.*, **67**, 1 (1955).
113. C. Jaccard and Levi, *Z. Angen. Math. Phys.*, **12**, 70 (1961).
114. G. A. Jeffrey and R. K. McMullan, *Prog. Inorg. Chem.*, **8**, 43 (1967).
115. G. A. Jeffrey, *Acc. Chem. Res.*, **2**, 344 (1969).
116. K. Johansson and J. C. Eriksson, *J. Coll. Inter. Sci.*, 1972.
117. F. H. Johnson, H. Eyring, and M. J. Polissar, *The Kinetic Basis of Molecular Biology*, Wiley, N.Y., 1954, 874 pp.
118. G. A. Johnson and S. M. Neale, *Koll. Zeit. Zeit. Polymere*, **185**, 130 (1962).
119. G. A. Johnson, S. M. A. Lecchini, E. G. Smith, J. Clifford, and B. A. Pethica, *Disc. Far. Soc.*, **42**, 120 (1967).
120. B. Kamb, *Structural Chemistry and Molecular Biology*, A. Rich and N. Davidson, Ed., p. 507, Freeman & Co., 1968.
121. A. V. Karyakin, G. V. Maisuradze, and G. A. Muradova, *Russ. J. Phys. Chem.*, **44**, 1179 (1970).
122. J. L. Kavanau, *Water and Solute-Water Interactions*, Holden-Day, San Francisco, 1964, 101 pp.
123. J. E. D. Kerr, Ph.D. Dissertation, University of Miami, 1970, 340 pp.
124. I. M. Klotz, *Science*, **128**, 815 (1958).
125. G. P. Kresiman and S. I. Chan, *Biopolymers*, **10**, 159 (1971).
126. J. Kumamoto, J. K. Raison, and J. M. Lyons, *J. Theoret. Biol.*, **31**, 47 (1971).
127. J. Kumamoto.
128. I. D. Kuntz, *J. Am. Chem. Soc.*, **93**, 514 (1971a).
129. I. D. Kuntz, *J. Am. Chem. Soc.*, **93**, 516 (1971b).
130. B. D. Ladbrooke and D. Chapman, *Chem. Phys. Lipids*, **3**, 304 (1969).
131. L. Landau, *Phys. Z. Sowjet*, **11**, 545 (1937).
132. L. D. Landau and E. M. Lifshitz, *Statistical Physics*, Pergamon Press, Ltd., London, 1958, 484 pp.
133. B. Lavenda, *J. Theoret. Biol.*, **31**, 395 (1971).
134. Korson, L., F. J. Millero and W. Drost-Hansen, *J. Phys. Chem.*, **73**, p. 34–37, 1969.
135. S. Lewis, *J. Theoret. Biol.*, **26**, 481 (1970).
136. S. Lewin, *Nature New Biology*, **231**, 81 (1971a).
137. S. Lewin, First European Biophysics Congress, Baden, Austria, p. 471, 1971b.
138. S. Lifson, Proceedings, XIVth Solvay Conference, Brussels, Belgium, Wiley/Interscience, p. 225, 1971.
139. G. I. Likhtenshtein, *Biofizika*, **11**, 24 (1966).
140. G. N. Ling, *A Physical Theory of the Living State: The Association-Induction Hypothesis*, Blaisdell Publishing, N.Y., 1962, 680 pp.
141. G. N. Ling, *Ann. N.Y. Acad. Sci.*, **125**, 401 (1965).
142. G. N. Ling, *Int. Rev. Cytology*, **26**, 1 (1969).
143. L. B. Loeb, *Static Electrification*, Springer-Verlag, Berlin, 1958, 240 pp.

144. P. F. Low, *Adv. Agron.*, **13**, 269 (1961).
145. I. Lubezky, F. A. Bettelheim, and M. Folman, *Trans. Far. Soc.*, **63**, 1794 (1967).
146. R. Lumry and S. Rajender, *Biopolymers*, **9**, 1125 (1970).
147. B. Lupas and T. Wilczok, *Biopolymers*, **10**, 1267 (1971).
148. S. Martin-Löf and C. Söremark, *Arch. Oral Biol.*, **16**, 873 (1971).
149. K. Marushige and J. Bonner, *Proc. Nat. Acad. Sci.*, **68**, 2941 (1971).
150. W. Matron, G. Ebert, and F. H. Müller, *Koll. Zeit. Zeit. f. Polymere*, **248**, 986 (1971).
151. R. L. McIntosh, *Dielectric Behavior of Physcially Adsorbed Gases*, Marcel Dekker, N.Y., 1966, 160 pp.
152. K. Mendelssohn, *Physics Today*, Clarendon Press, p. 46, 1969.
153. M. S. Metsik and O. S. Aidanova, *Research in Surface Forces*, **2**, B. V. Derjaguin, Ed., p. 169, Plenum Press Consultants Bureau, 1966.
154. A. P. Minton, *Trans. Far. Soc.*, **67**, 165 (1971).
155. J. Muir, *Trans. Far. Soc.*, **50**, 249 (1954).
156. E. J. Murphy, *J. Coll. Inter. Sci.*, **32**, 1 (1970).
157. T. Nakee, *J. Dairy Sci.*, **54**, 1780 (1971).
158. Anonymous, *Nature*, **225**, 316 (1970).
159. E. G. Nekrasova, I. V. Zhilenkov, and V. M. Federov, *J. Struct. Chem.*, **9**, 340 (1968).
160. S. S. Nelson and I. Blei, Research & Development Progress Report No. 221, U.S. Dept. of the Interior, p. 88, 1966.
161. A. Odajima, *J. Phys. Soc. Japan*, **14**, 308 (1959).
162. J. F. Padday, *Thin Liquid Films and Boundary Layers*, Special Discussions of the Faraday Society, p. 64, 1970.
163. C. Y. C. Pak and N. L. Gershfeld, *Nature*, **214**, 888 (1967).
164. L. S. Palmer, *Proc., Roy. Soc.*, **B25**, 674 (1952).
165. L. S. Palmer, A. Cunliffe, and J. M. Hough, *Nature*, **170**, 796 (1952).
166. L. Pauling, *Hydrogen Bonding*, D. Hadzi, Ed., Pergamon Press, N.Y., 1959, 571 pp.
167. J. W. Perram and S. Levine, *Molec. Phys.*, **21**, 701 (1971).
168. G. Peschel, *Zeit. f. Phys. Chem. Neue Folge*, **59**, 27 (1968).
169. G. Peschel and K. H. Aldfinger, *J. Coll. Inter. Sci.*, **34**, 505 (1970).
170. G. Peschel and K. H. Adlfinger, *Zeit. f. Naturf.*, **26a**, 707 (1971).
171. M. C. Phillips and D. Chapman, *Biochem. Biophys. Acta*, **75**, 301 (1968).
172. D. Pörschke, *Biopolymers*, **10**, 1989 (1971).
173. P. L. Privalov, First International Conference on Calorimetry and Thermodynamics, Warsaw, Poland, 1969, 12 pp.
174. P. L. Privalov and O. B. Ptitsyn, *Biopolymers*, **8**, 559 (1969).
175. P. L. Privalov and Y. I. Tiktopulo, *Biophys.*, **14**, 16 (1969).
176. P. L. Privalov, Y. I. Tiktopulo, and I. N. Serdyuk, *Biofizika* (*Engl. Trans.*), **14**, 1022 (1969).
177. P. L. Privalov and Tiktopulo, *Biopolymers*, **9**, 127 (1970).
178. W. E. Putnam and J. R. Kilpatrick, *J. Chem. Phys.*, **27**, 1075 (1957).
179. S. A. Rice, Proceedings, XIVth Solvay Conference, Brussels, Belgium, p. 243, 1969.
180. L. Rimai, J. T. Hickmott, Jr., T. Cole, and E. B. Carew, *Biophys. J.*, **10**, 20 (1970).
181. J. R. Robinson, *World Rev. Nutrition & Diet.*, **12**, 172 (1970).
182. B. Rosenberg, G. Kemeny, R. C. Switzer, and T. C. Hamilton, *Nature*, **232**, 471 (1971).
183. S. Ruthberg and L. Frenkel, *J. Res. Nat. Bur. Stan.*, **68A**, 173 (1964).
184. G. J. Safford, *Cryobiology*, **3**, 32 (1966).
185. N. J. Salsbury, A. Darke, and D. Chapman, *Chem. Phys. Lipids*, **7**, 9 (1971).
186. Santamaria and M. Tschapek, *9th International Congress of Soil Sciences Transactions*, **1**, #56 (1968).
187. T. Schleich, B. J. Blackburn, R. D. Lapper, and I. C. P. Smith, *Biochem.*, **11**, 137 (1972).
188. J. A. Schufle and N. T. Yu, *J. Coll. Inter. Sci.*, **26**, 305 (1968).
189. J. A. Schufle, W. Drost-Hansen, and C. T. Huang, *J. Coll. & I. Sci.* **54**, p. 184, 1976.
190. J. H. Schulman and R. Teorell, *Trans. Far. Soc.*, **34**, 1337 (1938).
101. A. Sheludko, *Colloid Chemistry*, Elsevier Publishing, N.Y., 1966, 277 pp.
192. J. C. W. Shepherd and E. H. Grant, *Proc. Roy. Soc.*, **307**, 335 (1968a).
193. J. C. W. Shepherd and E. H. Grant, *Proc. Roy. Soc.*, **307**, 345 (1968b).

194. J. Shereshefsky and C. Carter, *J. Am. Chem. Soc.*, **72**, 3682 (1950).
195. T. Sipos and J. R. Merkel, *Biochem.*, **9**, 2766 (1970).
196. E. W. Small and W. L. Peticolas, *Biopolymers*, **10**, 69 (1971a).
197. E. W. Small and W. L. Peticolas, *Biopolymers*, **10**, 1377 (1971b).
198. R. Söremark, C. Söremark, S. Martin-Löf, and N. Myrberg, *Annals, N.Y. Acad. Sci.*, **204**, p. 169, 1973.
199. T. C. Spelsburg, *Biochem. Biophys. Acta*, **228**, 550 (1971).
200. J. Stenesh, N. Schechter, P. Y. Shen, and C. Yang, *Biochem. Biophys. Acta*, **228**, 259 (1971).
201. A. Szent-Györgyi, *Bioenergetics*, Academic Press, N.Y., 1957, 143 pp.
202. A. Szent-Györgyi, *Persp. Biol. Med.*, Winter, 239 (1971).
203. G. Talsky, *Angew. Chem. Inter. Edit.*, **10**, 548 (1971).
204. G Talsky, Personal Communication, 1971.
205. H. Thürn, *Materialprüf*, **5**, 114 (1963).
206. H. Trauble and D. H. Haynes, *Chem. Phys. Lipids*, **7**, 324 (1971).
207. G. V. Troitskii, V. P. Zav'yalov, and I. F. Kiryukhin, *Biokhimiya*, **36**, 1107 (1971).
208. M. Tschapek, I. Natale, and R. Santamaria, *Koll. Zeit. Zeit. f. Polymere*, **211**, 143 (1966a).
209. M. Tschapek and I. Natale, *Koll. Zeit. Zeit. f. Polymere*, **211**, 146 (1966).
210. M. Tschapek, R. Santamaria, and I. Natale, *Electrochim. Acta*, **14**, 889 (1969).
211. A. R. Ubbelohde, *Disc. Far. Soc.*, **21**, 137 (1956).
212. A. R. Ubbelohde, *Melting and Crystal Structure*, Clarendon Press, Oxford, 1965, 325 pp.
213. A. R. Ubbelohde, Proceedings, XIVth Solvay Conference, Brussels, Belgium, p. 111, 1971.
214. J. H. van der Waals and J. C. Platteeuw, *Adv. Chem. Phys.*, **2**, 1 (1959).
215. C. Van Duijn, Jr., and J. H. Von Lierop, *Nature*, **5055**, 1313 (1966).
216. P. H. Von Hippel and T. Schleich, *Structure and Stability of Biological Macromolecules*, S. N. Timasheff and G. D. Fasman, Eds., Marcel Dekker, N.Y., 1969.
217. J. A. Walter and A. B. Hope, *Prog. Biophys. Molec. Biol.*, **23**, J. A. V. Butler and B. Noble, Eds., p. 1, Pergamon Press, 1971.
218. J. A. Walter and A. B. Hope, *Aust. J. Biol. Sci.*, **24**, 497 (1971b).
219. G. Wursuhn, *Naturw.*, **54**, 27 (1967).
220. P. M. Wiggins, *J. Theoret. Biol.*, **32**, 131 (1971).
221. A. S. Wightman, *Statistical Mechanics at the Turn of the Decade*, E. G. D. Cohen, Ed., p. 1, Marcel Dekker, N.Y., 1971.
222. J. J. Windle and T. M. Shaw, *J. Chem. Phys.*, **25**, 435 (1956).
223. D. E. Woessner and J. R. Zimmerman, *J. Phys. Chem.*, **67**, 1590 (1963).
224. A. P. Zhilenkov, Ph.D. Dissertation, Institute of Physical Chemistry of the Academy of Sciences of the U.S.S.R., 1963 (Abstract).
225. J. R. Zimmerman and J. A. Lasater, *J. Phys. Chem.*, **62**, 1157 (1958).
226. Y. Zotterman, *Handbook of Physiology: Neurophysiology*, J. Field, Ed., American Physiological Society, Washington, p. 431, 1959.

## Note

This paper was prepared in the fall of 1971 for presentation at the XV Solvay Conference in Brussels 1972. Since that time, a number of important papers have appeared, both regarding water structure in general and interfacial aspects of aqueous solutions. Below is presented a brief annotated bibliography of some of the more important papers.

Chapter II—Ben Naim, Arieh, "Water and Aqueous Solutions" (Introduction to Molecular Theory), Plenum Press, N.Y., 1974. This volume details the statistical mechanical studies by Ben-Naim on the structure of water and simple aqueous solutions, stressing hydrophobic interactions.

Franks, Felix, Editor, "Water—A Comprehensive Treatise," Plenum Press, 1972–1975. This monumental work, in five volumes, is an authoritative overview of the state of water and aqueous solution structure dealing with the physics and physical chemistry of water, ice, crystalline hydrates, aqueous solutions of electrolytes and non-electrolytes, aqueous solutions of macromolecules and (Vol. 5) water in dispersed systems.

Kay, Robert, Editor, "The Physical Chemistry of Aqueous Solutions," Plenum Press, N.Y., 1973. Proceedings from a symposium on the physical chemistry of aqueous systems, dedicated to Professor Henry S. Frank. Also printed as issues #2 and #3 of Vol. 2, Journal of Solution Chemistry.

Hobbs, Peter V., "Ice Physics," Oxford University Press, 1974.

Whalley, E., S. J. Jones and L. W. Gold., Eds., "Physics and Chemistry of Ice," Royal Society of Canada, Ottawa, 1973. Proceedings of symposium on the physics and chemistry of ice. This volume provides an overview of the current state of study of the structure and properties of ice.

Scheraga *et al.* For the many contributions by Scheraga to theories of water structure see: A. T. Hagler, *J. Phys. Chem.*, **76**, 3229–3243, 1972; B. R. Lentz *et al.*, *J. Phys. Chem.*, **78**, 1531–1550, 1974; L. L. Shipman *et al.*, *J. Phys. Chem.*, **78**, 2055–2060, 1974; and *J. Phys. Chem.*, **79**, 380, 1975.

Chapter III. Deryaguin, B., "Research in Surface Forces," Vol. 4, Consultants Bureau, New York, 1975. Contributed papers to the fourth conference on "Surface Forces in Thin Films and Dispersed Systems."

Matijević, E., Editor, "Surface and Colloid Science," John Wiley and Sons, New York. See particularly:

Dukhin, S. S. "Dielectric Properties of Dispersed Systems," loco cit. Vol. III, 1970.

Iler, R. K., "Colloidal Silica," loco cit., Vol. VI, 1973.

Dukhin, S. S. and B. V. Deryaguin, loco cit., Vol. VII, 1974. This volume is devoted to electrokinetic phenomena and equilibrium double-layer properties.

Lee, H. B., M. S. Jhon and J. D. Andrade, Journal of Colloid and Interface Science, Vol. 51, pp. 225–231, 1974. The first of a series of papers on the nature of water in synthetic hydrogels.

Jhon, N. S. and J. D. Andrade, Journal of Biomedical Materials Research, Vol. 7, pp. 509–522, 1973.

Metzik, M. S., *et al.*, Journal of Colloid and Interface Science, Vol. 43, pp. 662–669. A review of newer data on the structure and properties of water adjacent to mica surfaces.

Chapter IV. Wiggins, Phillppa, M., Medical Hypothesis, Vol. 1, 1975; see also Clin. & Exp. Pharm. & Physiol., 2, p. 171, 1975. In these papers Wiggins reports measurements on the ion distribution in narrow pores of silica gel. Specifically, Wiggins observed enhanced concentration of potassium ion in vicinal water and the exclusion of sodium ion—simulating the behavior of cellular systems. Furthermore, this ion distribution was found to be temperature sensitive, exhibiting sharp peaks in the potassium/sodium ratio at or near 15, 30 and 45°, in excellent agreement with the temperatures of thermal anomalies discussed in this paper.

Ling, Chaur-sun and W. Drost-Hansen, In: "Adsorption at Interfaces," edited by K. L. Mittal, American Chemical Society Symposium Series, Vol. 8, ACS, Washington, pages 129–156, 1975. Thermal anomalies are reported in the properties of water contained in porous glass as determined by differential thermal analysis (DTA).

Braun, C. and W. Drost-Hansen, Anomalous specific heats of water in porous materials (glass, zeolites, and activated charcoal) as determined by differential scanning calorimetry (DSC).

Drost-Hansen, W., In: "Chemistry and Physics of Aqueous Solutions," Symposium Proceedings, edited by W. A. Adams, *et al.*, (Proceedings from Electrochemical Society Meeting, Toronto, Canada, 1975), pp. 233–256. This paper deals with anomalous temperature (and pressure) dependencies of gas solubilities.

Clifford, J., In: Franks, "Water—A Comprehensive Treatise," loco cit, Vol. V, 1975, pp. 75–132. This paper is an authoritative review of the properties of water in capillaries and thin films.

Phillips, M. C., In: Franks, "Water—A Comprehensive Treatise," loco cit., Vol. V, 1975, pp. 133–172. A detailed review of the hydration and stability of foams and immulsions, with some consideration of the role of interfacial water in determining the stability of dispersed systems.

Ballario, C., A. Bonincontro, C. Cametti and S. D'Agostino, Nuovo, Cimento, Vol. 6, pp. 611–616, 1973. A dielectric study of aqueous suspensions of polystyrene particles.

Ballario, C., A. Bonincontro and C. Cametti; Journal of Colloid and Interface Science, Vol. 51, pp. 191–195, 1975. Study of dielectric losses in aqueous suspensions of bacterial spores.

Lobos, Z. J. and L. E. St'Pierre, Journal of Colloid and Interface Science, Vol. 51, pp. 196–199, 1975. Evidence is presented for thermal anomalies in interfacial tension of bovine serum albumin solutions.

Vignes, M. and K. M. Dijkema, Journal of Colloid and Interface Science, Vol. 49, pp. 165–172, 1974. The paper describes a role for vicinal water in determining the nature and extent of freezing of water in dispersed media by an ingenious technique, first proposed by Everett.

Chapter VI. Drost-Hansen, W., "Effects and Methods of Control of Thermal Discharges," Part 2, Serial #93–14, Senate Committee Print, November 1973; US Govt. Printing Office, Washington, D.C., pp. 847–1140. This paper deals with aspects of the relationship between temperature and aquatic chemistry, particularly the effects of temperature on ecology.

Chapter VII. Hazlewood, C. F., Ed., "Physico-Chemical State of Ions and Water in Living Tissues and Model Systems," Annals of NY Acad. of Sciences, Vol. 204, pp. 1–631, 1973. Contributed papers dealing with the subject matter of title.

Precht, H. and J. Christopherson, H. Hensel and W. Larcher, "Temperature and Life," Springer-Verlag, NY, 1973. A monumental update of the original volume "Temperature und Leben." An authoritative, exhaustive compilation of effects of temperature on living systems.

Drost-Hansen, W., "Structure and Functional Aspects of Interfacial (Vicinal) Water as Related to Membranes in Cellular Systems." Paper presented at: C.N.R.S. Meeting, "Participation—Energetique de L'aux Solvant Aux Interactions Specifique Dans les Systems Biologiques," Roscoff, France, Edition du C.N.R.S., #246, p. 177, 1976.

Kuntz, I. D. and W. Kauzmann, "Advances in Protein Chemistry," Vol. 28, pp. 239, 1974. An exhaustive review of protein hydration.

Cooke, R. and I. D. Kuntz, "Annual Review of Biophysics," Vol. 3, pp. 95–126, 1974. A review of the properties of water in biological systems.

Nishiyama, I., Research Bulletin, #110; Hokkaido National Agricultural Experimental Station, Hokkaido, Japan, 1975. This paper discusses "further evidence for thermal anomalies around 15, 30, 45 and 60°C in plants."

Karmas, E., Journal of Food Science, Vol. 38, pp. 735–739, 1973. The paper discusses water in biosystems.

Gamaley, I. A. and A. B. Kaulin, Physiological Chemistry and Physics, Vol. 6, pp. 445–456, 1974. The paper discusses aspects of the properties of water in muscle.

## Discussion of the Report of W. Drost-Hansen

*C. P. Bean* I would like to describe two experiments on the structure of water at silicate surface. The structure investigated is the long range type (100 Å–1000 Å) invoked by Prof. Drost-Hansen in his explanation of various experiments. Were Prof. Derjaguin here, I am sure he would refer to the extensive series of varied investigations[1] that have led him to the conclusion that a highly viscous proto-icelike structure extends for roughly 1000 Å from all charged surfaces in contact with pure water. It is difficult to overestimate the importance of the question of the existence of this structure. The interpretation of transport in all heterogeneous systems—especially biological systems—would be basically modified, were the phenomenon general.

The experimental system we have employed is that of a system of uniform, parallel pores through a thin sheet of muscovite mica. They are made by the so-called etched particle track process.[2] In brief, heavy ions are caused to pass through a thin ( ~ 5$\mu$) sheet of mica. The sheet is subsequently etched in hydrochloric acid. Initially pores of 70 Å are created along each trail of damage created and subsequent etching enlarges these pores to any desired degree.[3] The first experiment merely measures the temperature dependence of the resistance of a group of 70 Å pores filled with 0.1N KCl. (Concomitant streaming surface charge is negligible). The resistance is compared to that of the bulk bathing solution. These two resistances have the same temperature dependence. The activation energies are the same within 0.4%. This simple experiment shows no difference between ionic transport through these fine pores and that in the bulk of the solution. In particular, there is no evidence of ice formation within the pore as the temperature approaches 0°C. While this result is in apparent disagreement with the one cited by Drost-Hansen, it might be expected by Derjaguin who emphasized that pure water showed the structuring effects most emphatically. To investigate transport in pure water, we have measured diffusion of pure tritiated water through another system of pores.[4] The use of the usual diffusion constant gave one effective diameter while the change of transport with pressure assuming usual viscosity gave another and electron micrography gave a third. The conclusion of these and other experiments is that diffusion and transport of water through fine mica pores are such that strongly ordered water cannot be considered to extend more than 10 Å from the silicate surface.

If these results are general, they should perhaps occasion a further movement of Prof. Drost-Hansen's anomalies from the bulk where they originally existed, past their present 1000 Å range to a possible region of a few molecular layers on a surface.

## References

1. B. V. Derjaguin in "The State and Movement of Water in Living Systems," Academic Press, New York, 1965, p. 55.
2. *Phys. Rev. Letters*, **8**, 217 (1962).
3. C. P. Bean, M. V. Doyle, and G. Entine, *J. Appl. Phys.*, 41, 1454 (1970).
4. C. P. Bean in "Membranes, A Series of Advances" (G. Eisenman, ed.), Marcel Dekker, New York, 1972, p. 1.

*W. Drost-Hansen* Dr. Bean's observation of essentially unchanged conduction in the very narrow pores of his radiation + etch created membranes is somewhat surprising. Other measurements, especially by Prof. Schufle and co-workers in New Mexico.[1–2] have suggested very large differences in the conduction of dilute electrolytes in narrow capillaries.

Using glass (or quartz) capillaries, ranging from 5 microns to several thousand microns in diameter values, the apparent energy of activation $\Delta E_A^*$ for ionic conduction of dilute solutions of KCl and HCl increases rapidly with decreasing diameter (and with decreasing concentration). Values for $\Delta E_A^*$ varies by as much as a factor of 2 from the corresponding bulk values.

On the other hand, some measurements of ionic conduction in porous leached Vycor (with average diameter of 40 Å have failed to reveal any anomalies between 20° and 40°C (measured at 1 or 2°C intervals). No anomalies were observed for $10^{-2}$ M solutions of the chlorides of Li, Na, K, Rb and Cs. However, for $10^{-2}$ M solutions of $R_4N^+$ chlorides, anomalies do appear to occur near 30°C for R = butyl, and possibly propyl.

Regarding the absence of thermal anomalies under certain circumstances—namely, for very narrow pores in mica (Mr. Bean's experiments) or in silica (Vycor) (our own experiments)—note that Peschel and Aldfinger[1] found far less distinct temperature anomalies in the disjoining pressure for plate separations of 100 Å, compared to larger separations (300 and 500 Å). One might perhaps venture the guess that very close to certain types of surfaces (perhaps only ionic or strongly polar solids) the type of extensive, weak orientations I discussed before may not readily be established.

## References

1. J. A. Schufle and Yu Nai-teng, *J. Coll. and Interface Science*, **26**, 305–316, 1968.
2. J. A. Schufle, Chin-tsung Huang, and W. Drost-Hansen, see ref. 189, p. 336.

*C. P. Bean* With regard to the electrical impedance of fine electrolyte filled pores, it is necessary to exercise some care to minimize the effect of internal surface charges. The experiment alluded to in my earlier comment had, as judged from streaming potential measurements, almost zero surface potential. In addition, the solution in the pores was 0.1N KCl. Use of this relatively strong solution minimizes any residual surface conductance effects.

Neglect of these precautions, through the use of dilute solutions and highly charged surfaces, will give a system that is similar to an ion exchange membrane. The temperature dependence of the conductance of such a system may be a quite complex function of temperature. To ascribe this complexity to states of water can be most misleading.

## Reference

1. G. Peschel and K. H. Adlfinger, *Zeit. fur Naturforschung*, **26a**, 707–715, 1971.

*S. Claesson* I would like to talk about the transitions in cellulose. I happen to know a little about this, because this transition, which is a second order, was first discovered by Prof. Wahba in Cairo, by measuring the density of different cellulosic materials as a function of temperature. Everybody was very suspicious, because the change in slope was small. But anyhow, the cellulose people became very interested in it, repeated the experiment many times and got very good values. The experiments were taken up by the Cellulose Research Inst. of Stockholm and also by the Pulp and Paper Res. Inst. in Canada.

They were repeated in different ways. The people in Stockholm developed an extremely sensitive way of determining the density of thin films by shooting a beam of electrons through the film from radioactive source, measuring the intensity with high precision and using a refined counting technique.

If the film would contract, it would get denser, and if it would expand, it would get less dense.

They did find a transition also at −16°C. The two transitions have been established even better by another technique, the torsion pendulum. They took a small fibre of cellulose (1 mm long or little less and 0.1 mm of diameter) and they did the torsion pendulum to work as function of the temperature; because you would expect, if there is something happening to your fibre of cellulose, to get perhaps bigger effects in mechanical measurements. In this way again, they could get more proof about this transition point and again, as one would expect from mechanical experiments, they got a much bigger change in damping at the transition point than they did in frequency. The reason is that cellulose in these experiments contains some water (about half a molecule of water/glucose unit) and that forms a very strong coupling between the cellulose chains. If these are weaker, you would expect it could be easier for a little water to move away and then it would be less damping. This has also been confirmed by work from NMR measurements where they have been looking at the water in the cellulose and they have actually found in this region of water content, a very marked broadening of the lines which again proves that you have a strong lateral coupling in the cellulose system.

*A. R. Ubbelohde* Over what sort of distances would any orientation of water molecule need to be inferred?

*S. Claesson* There is no free water present here. This corresponds to what is called half hydrate where you have about half a molecule/glucose unit. When you add water, you have more or less well order; you get weaker coupling in certain region which are reported to be the amorphous region of the transition.

*A. R. Ubbelohde* When you add more water, does the transition disappear?

*S. Claesson* It is difficult to say, because all these experiments have been made with material with constant water content.

*M. Magat* I have no experience concerning possible transitions in thin layers of water, however, I maintain my old statement that there is a rapid change of the water structure between say 30°C and 50°C. This is based on the following facts:

1) As Walrafen has shown (Fig. 4 of my report) there is an inflection point in the curve giving the intensity of intermolecular Raman bands as function of temperature.

2) $Cp$ shows a minimum in the same temperature region[1] and $Cv = f(T)$, if carefully inspected, shows a slight change of slope.

3) The second maximum of the X-ray diffraction curves, that exists at 25°C, practically disappears at 50°C as observed by Steward and others in the twenties[2] and by Narten *et al.*[3] more recently.

4) There is an extremun around 40°C in the expansion and in compressibility coefficients.[4]

5) The expansion coefficient as function of pressure changes its slope at 40°C.[5]

However no anomaly appears in dynamic properties. This would mean that it is essentially the $\mathscr{D}$ structure but not so much the $\mathscr{V}$ structure that shows "anomalies" in the temperature region concerned. These "anomalies" reflecting a structure evolution, may be important in biology because they fall in the temperature range of warm-blooded animals.

Finally I would like to call to your attention the last slides shown by Dr. Drost-Hansen. These slides show that a rapid growth of some aquatic organisms set in very suddenly, over a very narrow temperature region. This is extremely important for the thermal water pollution problem. It is usually tacitly assumed that the rate of growth and of developement of such organisms is a continuous, although exponential function of temperature. If it were so, a small increase, of say 2–5°C, of the water temperature would be of relatively small importance. If however the effect of temperature is discontinuous, such a temperature increase may mean an extreme difference in ecological conditions. We should never forget that if the present rate of thermal or nuclear production of energy is maintained, the temperature of the Rhine will reach before the end of the century 35°C and will show no more seasonal variation. This is an example that we all have to meditate.

## References

1. D. Eisenberg and W. Kauzmann. The structure and properties of water. Oxford, Clarendon Press, 1969, p. 172.

2. G. W. Stewart, *Phys. Rev.*, 37, 9, 1931.
3. A. H. Narten, M. D. Danford, and H. A. Levy, *Disc. Far. Soc.*, 44, 97, 1967.
4. D. Eisenberg and W. Kauzman, *loc. cit.*, p. 184.
5. D. Eisenberg and W. Kauzman, *loc. cit.*, p. 186.

*W. Drost-Hansen* I certainly agree with Professor Magat that the existence of abrupt changes in the responses of cellular systems to temperature (for instance around 29° to 32°C) will have far reaching impact on ecology, and for that reason is of prime importance in connection with the need to control thermal pollution.

*M. Mandel* There are two questions I would like to ask:

1) In as far as the thermal anomalies of water are concerned, I wonder if analogous measurements have been performed on other liquids and solutions, and if so, what results have been obtained especially concerning possible anomalies.

2) If it is true that the stabilization of water structures are due to the possibility of large energy fluctuations being absorbed by the solid at the interface, how do you explain the stabilization of these structures at a water/vapor interface?

*W. Drost-Hansen* As indicated, the thermal anomalies are remarkably sharp—often extending over an interval of less than 3°C. Hence, to detect and delineate the thermal anomalies it is necessary to make measurements over large temperature ranges at closely spaced temperature intervals. Such measurements have not often been carried out on non-aqueous, large surface-to-volume ratio systems. Hence, I cannot state what the likelihood is of finding thermal anomalies in properties of non-aqueous disperse systems.

As far as the pure air/water interface is concerned—very far from any solid interface—I do agree with Prof. Randles that no thermal anomalies are observed. However, only slight amounts of a second "component" may induce structuring of the type giving rise to thermal anomalies. A monolayer or even only a partial monolayer at the air/water interface may give rise to notable anomalies. Prof. Cini and co-workers[1] (in Florence) have shown thermal anomalies in the surface tension of very dilute solutions of heptanol in water, but these anomalies disappeared completely when activated charcoal was added to the system, removing the last trace of heptanol (by adsorption). The evidence for thermal anomalies in the properties of "slightly impure" aqueous surfaces will be reviewed in some detail in a forthcoming paper[2] from a conference on air/sea interactions. Partial coverage of the open ocean air/sea interface may play a role in the origin of tropical hurricanes, which are generally acknowledged to be generated only when the temperature of the sea surface exceeds 30°C!

*J. E. B. Randles* The results of the vibrating capillary experiments seemed to be very-well defined. Have you done similar experiments on hydrogen bonded liquids other than water, and on non hydrogen-bonded liquids?

With regard to Dr. Drost-Hansen's explanation of the stabilization of water structure near to an interface with a solid, I doubt whether it is tenable. A solid adjacent to the liquid will not damp out local energy fluctuations, any more than will adjacent liquid. These statistical fluctuations depend on the average thermal energy, or temperature, which will not be changed.

## References

1. R. Cini, G. Loglio, and A. Ficalbi, *Nature* **223**, 1148–49, 1969.
2. W. Drost-Hansen, *J. Geophysical Research*, **77**, 5132–5146, 1972.

*W. Drost-Hansen* As far as I remember, Kerr tried one non-polar liquid in the "vibrating hair pin capillary" and as would be expected, did not find any evidence for thermal anomalies. Unfortunately, however the use of this instrument is not as satisfactory as one might have hoped for. Hysteresis effects occur and there are many experimental difficulties.

It seems that a far better approach is the use of the impressive device constructed by Professor Peschel for the disjoining pressure measurements.

With respect to the explanation of the paradoxical effect in terms of an "energy delocalization" I agree that this is only a very tentative notion. The explanation might also be in conflict with the principle of microscopic reversibility as mentioned in my report. However, I do believe that the paradoxical effect is real and hence an explanation is required! I certainly would welcome any other suggestions of possible mechanisms from the theoreticians.

*S. Claesson* I think one has to be very careful when talking about biological effects on small objects.

I would like to talk about very simple experiments people were doing some 20 years ago, when they used ultra-centrifugation and tried to determine the solvatation of particles which sedimented in the ultra-centrifuge. These particles were protein molecules or something like that. They would try to determine the density of these molecules by adding more and more substance to the solvent, so that its density would be the same as that of the particles and the particles would not sediment. It is obvious, that if you add heavy water, to make the solvent heavier, from the solvent concentration of heavy water, at no sedimentation you will find the specific volume of the particles and nothing else.

So they did add bigger particles and these experiments were repeated many times by different workers, who all made the same sad mistake: if you add something which is bigger, you don't get the correct density, because there is a local density around the proteins which is not what you measure macroscopically over the whole solution. Prof. McLaren in Berkeley did some very nice experiments in a similar way with enzymes. He took substrates of different size, this affects the local concentration; therefore this would also affect the possibility for the enzymes to attack small bodies or big bodies. He did find marked difference if he put substrates as small droplets or if he added them as bigger particles, and in the same way, he found differences if he used big acid molecules or small ones to define the pH.

One has to be careful when trying to look onto this kind of reaction between macromolecules when sizes are comparable to those of macromolecules themselves.

*W. Drost-Hansen* Prof. Claesson's warning is certainly appropriate. I might mention another example of somewhat similar nature, from some early experiment which (the late) Howard Neil and I carried out about 15 years ago.

Small droplets of water were produced in air and allowed to fall into a benzene–bromobenzene density gradient tube. In this gradient the droplets were distributed according to size—the smaller droplets sinking deeper than larger droplets (size range ~10$\mu$ to ~50$\mu$). This would suggest that the smaller the drop the higher the density—and the apparent difference was highly significant. We thought this was good evidence for relative enhancement of density of the water at the water/organic liquid interface; fortunately, we did not publish these results; not long after, it was recognized by someone in England that a gradient in interfacial tension also exist and this effectively gives rise to a size-dependent "boyant force." Making some reasonable assumptions regarding the values of the interfacial tension, corrections for the effect of this force essentially completely removed the "anomalous density increase" of the small droplets.

*D. H. Everett* Drost-Hansen is referring I think to the explanation first given by C. R. Burch who was at that time critical of the use of density gradient columns for the determination of the density of polyethylene crystals.

*S. A. Resing* There is a simple NMR experiment for finding the amount of water bound to a globular protein or silica sphere. Crudely the NMR relaxation times for a simple system are of the form

$$T_i^{-1} = \sigma_0^2 \tau$$
$$T_i^{-1} \sim \frac{\sigma_0^2}{i + \omega^2 \tau^2} \tag{1}$$

where $\tau$ is the turning or correlation time of the molecule, and $\sigma_0^2$ is the nuclear second moment which involves nuclear constants and geometrical factors.

Suppose that a molecule may sample two sites with occupation probabilities $P_b$ and $(1 - P_b)$ where $P_b$ is for instance the fraction of molecules bound to a protein surface in such a way that its correlation time is the lifetime in the bound state or the correlation time of the protein molecule, whichever is shorter. Under conditions of fast exchange, what is seen is the average relaxation rate as

$$T_i^{-1} = \frac{P_b}{T_{ib}} + \frac{1 - P_b}{T_{if}} \qquad i = 1, 2 \tag{2}$$

where the subscripts $b$ and $f$ refer to bound and free respectively. $P_b$ is then $kC$, where $C$ is the concentration and $k$ represents the weight of bound water per weight of protein.

Rearrangement and differentiation with respect to concentration give the equations

$$\frac{\partial}{\partial C}\left(T_i^{-1} - T_{if}^{-1}\right) = k\left(T_{ib}^{-1} - T_{if}\right) \qquad 1 = 1, 2 \tag{3}$$

The turning time of the protein is known from dielectric experiments to be about $10^{-8}$ sec, which makes

$$T_{ib} \ll T_{if}$$

The ratio of the slopes gives $T_{2b}/T_{1b}$, and for a given experimental NMR frequency $\omega$ it is possible to deduce $\tau$ by the use of Equ. (1). This value of $\tau$ is then used with $\sigma_0^2$ (intramolecular) for water and the $T_2$ equation, to give $T_{2b}$ which in turn is put back in (3) to give the fraction of bound water. In the case of bovine serum albumin, this is about 0.03 g/g—about one monolayer. The value of $\tau$ is $3 \times 10^{-8}$ cm, which is somewhat shorter than the shortest Debye time of the protein ($7.5 \times 10^{-8}$ sec). This implies that the bound water molecule stays on the protein for the order of the turning or Debye time of the protein.[1] These results of ours, rather preliminary, have since been *superseded* and developed by many others.

## References

1. H. A. Resing and J. J. Krebs, Abstracts of Papers, 140th Meeting of the American Chemical Society, p. 130 (1961).
2. S. Koenig and W. Schillinger, *J. Biol. Chem.*, **244**, 3283 (1968); **244**, 6520 (1969).
3. B. Blicharska, Z. Florkowski, J. Hennel, H. Held, and F. Noack, *Biochim. Biophys. Acta*, **207**, 381 (1970).