PHASE TRANSITIONS IN BIOLOGICAL SYSTEMS: MANIFESTATIONS OF COOPERATIVE PROCESSES IN VICINAL WATER *

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INTRODUCTION

The properties of water near interfaces, including biological interfaces, often appear to be notably different from the properties of bulk water. These differences must reflect dissimilar structures of vicinal water as compared to bulk water. There is evidence that the distance over which these anomalous structures are propagated into the liquid from the interface may be of the order of 0.1 μ—a distance comparable to those of interest in cellular biology. The types of structures which may become stabilized by proximity to an interface are discussed briefly in terms of high pressure ice polymorphs, clathrate hydrates, and others. Transitions between such different stabilized vicinal structures are expected to occur as temperature is changed, and the frequently reported thermal anomalies for aqueous vicinal systems (including cellular systems) are interpreted as higher order phase transitions in the vicinal water. Examples are presented of such thermal anomalies, and some speculations are made regarding the effects of vicinal structuring on various biological phenomena (such as growth optima and minima, body temperatures, and active transport).

STRUCTURE OF WATER

To date there is no satisfactory understanding of the structure of water. Two major rival theories of water structure coexist, namely the continuum, "uniformist" model and the mixture models. Due to the abundance of current reviews of water structure in the literature,[13a] this subject will not be discussed in this paper. Only one aspect will be stressed here: the cooperativity of hydrogen bonding.

Henry Frank first introduced the idea of cooperativity of hydrogen bonding in liquid water in 1958. The essence of Frank's suggestion is that when two neighboring water molecules form a hydrogen bond between them, the molecules essentially undergo an acid-base interaction (in the Lewis sense) with the result that one of the molecules becomes more acidic, and the other more basic (than if they had remained unbonded monomers). After this initial step, additional hydrogen bonds will then form with greater ease—one acidi-

cally, the other by a base mechanism—and thus a positive feedback results. The process may be extended into three dimensions and result in the formation of large clusters due to the cooperative nature of the bonding. It appears now from theoretical work [2,3] that there is good evidence for cooperativity in hydrogen bonding as deduced from ab initio quantum mechanical calculations. The cooperativity thus induced may then, in turn, impart the property to which Henry Frank has referred as "flickering clusters." Because of the cooperativity it is to be expected 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."

PHASE DIAGRAM OF WATER

At least 8 different high-pressure ice polymorphs exist, in addition to cubic Ice-I, amorphous ice and "glassy water." [12] It is of interest to note the energetics involved in the transitions between the different ice polymorphs. See TABLE 1. The most striking characteristic is the low lattice energy difference among the various polymorphs. Perhaps most surprising is the difference of only 19 calories per gram mole between Ice-I and Ice-II. More often, the energy differences amount to a few hundred calories per gram mole. In either case, these values are small by comparison with thermal energy. This, then, leads to the hypothesis that where "solid, structured water" is created, particularly under notably nonequilibrium conditions, the ice thus formed may be that which is kinetically favored rather than the form which is thermodynamically stable. We return to this in connection with cooperativity in the ice lattice, and possible structured entities of water near interfaces. The dielectric properties of ice are unusual: note that for the nonpolar dielectric polymorphs (Ice-II, VIII and IX) the dielectric constant is remarkably low, while the dielectric constant for the other proton-disordered ices exceeds 80.

CLATHRATE HYDRATES

In addition to the ice polymorphs, it is possible to construct rigid lattice networks, consisting primarily of water by the introduction of suitable (generally nonpolar) solutes. Thus, a large number of clathrate hydrates have been described. Jeffrey [14,15] has summarized some general features of organic clathrate hydrates. See TABLE 2. The pentagonal dodecahedral structure (and tetrakaidecahedra) found in the chlorine hydrate is only one example of the multifaceted types of clathrate hydrates possible.

THERMAL ANOMALIES

General Introduction

In view of the large number of structured forms of water which have been discussed (namely the clathrate hydrates and high-pressure ice polymorphs), it is reasonable to assume that some of these structures may occur

TABLE 1

THERMODYNAMICS OF ICE-ICE TRANSITIONS *

| Transition | | T | P | ΔV | ΔS | ΔH | ΔE | $P\Delta V$ |
From	To	(°C)	(kbar)	(cm³ 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 ±0.0005	~−0.93	−260	−260	—

* Values of ΔH, ΔE, and $P\Delta V$ have been calculated by the present author.
† Supercooled ice III.

preferentially in aqueous systems near interfaces. Details of such vicinal stabilization will be discussed later. The local stabilization of structured entities appears a very real possibility, and the types of structured water discussed above are likely candidates. Obviously, the vicinal, structured water must be expected to retain some of the attributes of liquid water rather than those of crystalline solids. Hence, it is hardly surprising—with a multitude of possible structured entities—that transitions should take place between these different types of structures. Most likely, structural changes can be induced by changes in temperature (and possibly by changes in pressure[9-11]). The structures are hardly space-filling, rigid, crystalline entities, but rather possess

TABLE 2

CLASSIFICATION OF ORGANIC HYDRATES *

	Structural Characteristics

Ices
   Hexagonal, cubic, high-pressure ices,
     II–VII
	Infinite three-dimensional hydrogen-bonded
	   framework structures.

Clathrate
hydrates   { gas hydrates
	Nonbonded guest molecules stabilize clath-
	   rate voids in polyhedral host structures of
	   hydrogen-bonded water molecules

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 polyhedral
	   clathrate voids occupied by hydrogen-
	   bonded alkylamine molecules.

Water framework structures
   Three-dimensional
   Two-dimensional
   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 func-
	   tion of the solute species.

* Hydrates ordered in the degree to which $(OH_2)_n$ is structure-determining.

attributes of the liquid state (perhaps better described as liquid crystals, or anticrystalline arrangements[25]). Thus, it is to be expected that transition points should not be sharp. On the contrary: such changes will more likely resemble higher-order phase transitions. Indeed, transitions have been observed although they have, in the past, not attracted the attention they appear to deserve.

Examples of Thermal Anomalies

A number of examples of thermal anomalies in the properties of water near interfaces are discussed briefly in this section. Attention is called to the

fact that it is claimed only that the thermal anomalies occur in the properties of water near interfaces, not in bulk solution of pure water or in ordinary aqueous solutions (particularly of low molecular weight electrolytes). The diffusion coefficient for thiourea in water has been shown to reveal anomalous temperature dependencies. The diffusion coefficients were measured by a unique and ingenious technique developed by Dreyer and associates.[4, 5] The authors noted that unusual, relatively abrupt changes occurred at a number of discrete temperatures; the temperatures at which Dreyer and colleagues observed such anomalies coincide fairly well with the temperatures the present author has advocated for a number of years as regions of higher order phase transitions in vicinal water. These temperature ranges are 14–16° C, 29–32° C, 44–46° C, and 59–62° C. Dreyer and coworkers stressed that the anomalies are likely manifestations of structural effects caused by the walls of the capillaries used.

The half-life of the vibrations of an oscillating, U-shaped quartz capillary tube filled with water (vibrating in a vacuum) depends in an unusual way on the temperature. Forslind[13] showed that the half-life of the vibrations goes through a maximum near 30°. More recently, Kerr and the present author[16] have repeated and confirmed the observations by Forslind. A tentative explanation of this effect has been presented recently.[10, 11]

Kerr[16] measured the electrical conduction in the capillaries used in connection with the vibrating U tube "viscometer," and found that the resistance as a function of temperature (for a $5 \cdot 10^{-2}$ M sodium chloride solution) exhibits a sharp minimum near 30° C. Although the capillary employed had a diameter of approximately 50 μ, a dimension which would ordinarily have been expected to reflect only bulk properties, independent of the nature and extent of the water/wall interactions, it was found that a notable anomaly does occur in the vicinity of 30–32° C. Evidence for anomalous changes in membrane resistances has been discussed by the present author in a number of papers.[6–8]

Peschel and Adlfinger[18] have devised an ingenious device for measuring disjoining pressures of liquids between two plates. With this instrument, these authors have measured the disjoining pressure as a function of temperature for different plate separations. Their results clearly demonstrate well-developed, sharp peaks in the disjoining pressure at approximately the temperatures enumerated previously by the present author. Furthermore, the distance at which a certain (arbitrary) value of disjoining pressure (10^4 dyn/cm²) is attained may approach 0.1 μ.

Dielectric studies of water near solute interfaces have been made by Shepherd and Grant;[23, 24] these authors studied the dielectric properties in the megacycle range of aqueous hydroxyproline and proline solutions. It was observed that the dielectric constant appeared to decrease abruptly in the vicinity of 20–30° C, and 30–40° C. The authors speculated that this might be due to the relatively abrupt disappearance of some structured entity of water of hydration of these amino acids in solution. Only rarely has reliable information been obtained, indicating anomalies in the properties of such relatively low molecular solutes. The primary importance of this study is in connection with the study of the nature of hydration of collagen, which is unique among the proteins in containing large fractions of proline and hydroxyproline.

Ballario and coworkers[1] have studied the dielectric properties of suspensions of nylon spheres in water. These authors concluded that a separate structured type of water exists below approximately 28° C, which disappears

between 28 and 32° C. Privalov and associates [19-22] have measured the specific viscosity of highly dilute collagen solutions as a function of temperature for various initial shear rates in a rotary viscometer. It was found that the behavior of these rather dilute solutions is apparently non-Newtonian and, for small shear rates, the solutions show a notable maximum in the vicinity of 30° C.[11] In summary, it appears that the structure of water near interfaces does indeed exhibit higher order phase transitions at a number of discrete temperatures.

Vicinal Water

In order to observe higher order phase transitions, it is necessary to have some element of cooperativity. It is suggested that this cooperativity is the result of extensive structuring of water adjacent to an interface. Note that no less than four thermal anomalies are observed, requiring the existence of at least five different types of structured water, with stabilities extending over relatively limited ranges of temperature. (Incidentally, it is possible that more than four thermal anomalies occur between the freezing and the boiling point, but the temperatures of other anomalies have, thus far, not been firmly established, due to the frequent lack of sufficiently closely spaced temperature data on interfacial systems.)

STRUCTURE OF WATER NEAR INTERFACES

Structure vs. Lifetimes

For a discussion of the structure of liquids, particularly in terms of structured entities and their lifetimes, see Eisenberg and Kauzmann (1969; loc. cit., p. 150ff). In terms of the present discussion, the question is essentially: are the vicinal structures essentially identical to those latently present in bulk, but merely enjoying notably enhanced lifetimes, or, alternatively, are the structures created by proximity to the interface, ab initio, from the bulk? This author favors the enhancement of latently existing structured elements, already present in the liquid water. In the bulk, these structures would have lifetimes characteristic of the flickering clusters, i.e., of the order of 10^{-11} sec. Near an interface, the lifetimes may be increased by one (or more) orders of magnitude.

Types of Structures

The nature of the structure of water immediately adjacent to a solid surface must depend on the specific nature of the surface. Thus, different types of solid/water interactions are expected in the case of an essentially nonpolar, polar, or ionic surface. Previously, these aspects were discussed in some detail;[7] it was suggested that the water adjacent to an ionic (and, to a lesser extent, to a dipole) surface were oriented by ion-dipole and dipole-dipole interactions. These interactions are not expected to give rise to any long-range ordering. The water adjacent to a nonpolar surface is expected to be differ-

ently structured. Possible structured elements in this connection include clathrate, cagelike structures.

It had been proposed earlier [7] that a disordered zone might exist between the highly structured water adjacent to an ionic (and possibly a dipolar) solid. The disordered zone is established as the result of a "lattice mismatch" between the highly ordered structures, induced by the solid, and the vastly different structure of bulk liquid. The evidence for the existence of a disordered zone is mostly indirect and tentative. Particularly near a nonpolar surface, there may be no disordered zone at all. The question remains unresolved.

A great deal of evidence is available from which estimates can be made as to the depth of changed water structures near solids. Probably, the dimensions of the structurally modified water layers depend somewhat on the specific nature of the solid with which the water is in contact. Elsewhere, however, evidence for the existence of a general, long-range ordering effect in water was reviewed in some detail.[8, 11] It appears that the distances affected may extend from one tenth to several tenths of a micron.

Paradoxial Effect

Thermal anomalies of the type discussed above appear to occur in the properties of water adjacent to various solids, more or less independently of the specific nature of the chemical composition of the solid. Thus, the temperatures at which thermal anomalies occur appear almost independently of whether the surface is hydrophobic or hydrophilic. Other evidence for the independence of the structure of vicinal water on the detailed chemical nature of the substrate has been discussed by this author elsewhere.[8, 11] The reader is referred to these articles for a more detailed account.

Liquid Crystals and Anticrystalline Structures

It is possible that the water adjacent to a solid may possess the characteristics referred to by Ubbelohde [25] as anticrystalline structures. These structures resemble liquid crystals, conceivably describable in terms of cybotactic swarms. The properties of such structures are probably noticeably different from those encountered in normal bulk liquid.

ENERGY DELOCALIZATION AS CAUSE OF THE PARADOXIAL EFFECT

The paradoxial effect (i.e., the notion that the temperatures of the thermal anomalies are independent of the detailed chemical nature of the solid with which the water is in contact) is one of the most remarkable observations that has resulted from the study of the thermal properties of vicinal water. It is proposed that this effect occurs as the result of an energy delocalization. Thus, near a solid surface, the water structures latently present in bulk water (assuming a mixture model for the structure of water) may gain temporal stability as the result of a delocalization effect. A thermal fluctuation, which would ordinarily have been sufficient to disrupt a certain critical hydrogen bond in a cooperative cluster of hydrogen-bonded water molecules, may

become dissipated over a number of vibrational (or possibly rotational) modes of the adjacent solid. Obviously, the solid interface would not continually acquire an excess energy input. It is conceivable, however, that the energy acquired by the solid could be redistributed over the vicinal water, but in amounts below the critical amount necessary to disrupt one of the (cooperative) hydrogen bonds in the vicinally stabilized structures. Although this concept is somewhat unsatisfactory, an explanation such as "energy delocalization effect" appears necessary to explain the independence of the temperatures of the thermal anomalies on the detailed chemical nature of the solid with which the water is in contact.

Large-scale cooperative effects have been observed at the ice/water interface. The freezing of dilute solutions of electrolytes has been shown to be associated with the generation of large potentials; this is the so-called Workman-Reynolds effect. The freezing of a 10^{-4} M potassium chloride solution may lead to potentials as high as 30 volts between the ice and the unfrozen, supernatant liquid. It appears that large-scale cooperative effects operate in this system, possibly involving the order of 10^5–10^6 water molecules per chloride ion incorporated. (For a discussion of the mechanism and energetics of this phenomenon, see other work by this writer.[6, 11])

This writer proposes that the origin of the freezing potentials is due to a lattice strain energy caused by the bond angle opening of the water molecule in passing from the liquid state to the nearly tetrahedral arrangement of ordinary hexagonal ice. The details of the mechanism involved in the generation of the freezing potentials remain obscure, but appear likely to involve the transient occurrence of layers of polar ice structures (such as Ice-II). This type of strongly polar layer of water may possibly resemble the idea of "polarized multilayers" introduced by Ling.[17]

It appears that the most characteristic aspects of vicinal water structures are the long range over which ordering is encountered and the low energetics accompanying this ordering. The energetics of various structured forms of water have been discussed in some detail elsewhere.[8, 11] It has been pointed out [12] that for most high-pressure ice polymorphs, the lattice energy differences range from 19–550 cal/g mol. For many of the ice polymorphs, the lattice energy differences are around 200 cal/g mol. Thus, vastly dissimilar structures of ice with noticeably different properties may exist, corresponding only to very minor differences in lattice energies. There is no reason to believe that a similar phenomenon may not take place for water vicinal to various types of solid interfaces. Indeed, it is such extensively ordered structures with low lattice energy differences that are particularly conducive to exhibit higher order phase transitions.

In biological systems, a large fraction of the water will be adjacent to various types of interfaces, ranging from interfaces with macromolecular solutes to membranes. If a general tendency exists for ordering of such vicinal water, it is not surprising that thermal anomalies are observed on many levels of cellular activity.[8, 11]

REFERENCES

1. BALLARIO, C., A. BONINCONTRO, C. CAMETTI & S. D'AGOSTINO. 1971. Dielectric loss of a nylon-water suspension in the microwave X band. Nature Phys. Sci. **234:** 179–180.

2. DEL BENE, J. & J. A. POPLE. 1969. Intermolecular energies of small water polymers. Chem. Phys. Lett. 4: 426–428.
3. DEL BENE, J. 1971. Theoretical study of open chain dimers and trimers containing CH_3OH and H_2O. J. Chem. Phys. 55: 4633–4636.
4. DREYER, G., E. KAHRIG, D. KIRSTEIN, J. ERPENBECK & F. LANGE. 1968. Grundlagen und Anwendungen einer neuen diffusionsmethode in flugsiger phase. Z. Naturforsch. 23a: 498–503.
5. DREYER, G., E. KAHRIG, D. KIRSTEIN, J. ERPENBECK & F. LANGE. 1969. Structural anomalies of water. Naturwiss. 56: 558–559.
6. DROST-HANSEN, W. 1967. The water-ice interface as seen from the liquid side. J. Coll. Inter. Sci. 25: 131–160.
7. DROST-HANSEN, W. 1969. On the structure of water near solid interfaces and the possible existence of long range order. Ind. Eng. Chem. 61: 10–47.
8. DROST-HANSEN, W. 1971. Structure and properties of water near biological interfaces. In Chemistry of the Cell Interface. H. D. Brown, Ed. B: 1–184. Academic Press. New York.
9. DROST-HANSEN, W. 1972. Effects of pressure on the structure of water in various aqueous systems. In SEB Symposium #26, The Effects of Pressure on Organisms. : 61–101. Cambridge Univ. Press.
10. DROST-HANSEN, W. 1972. Molecular aspects of aqueous interfacial structure. J. Geophys. Res. 77: 5132.
11. DROST-HANSEN, W. 1972c. Water at biological interfaces. Presented at XV Solvay Conf. Brussels, Belgium. To be published.
12. EISENBERG, D. & W. KAUZMANN. 1969. The Structure and Properties of Water. Oxford Univ. Press. New York.
13. FORSLIND, E. 1966. Rheology. Svensk Naturv. 2: 9–74.
13a. HORNE, R. A. 1972. Water in Aqueous Solutions: Structure, Thermodynamics and Transport Processes. Wiley-Interscience. New York.
14. JEFFREY, G. A. & R. K. McMULLAN. 1967. The clathrate hydrates. Prog. Inorg. Chem. 8: 43.
15. JEFFREY, G. A. 1969. Water structure in organic hydrates. Acct. Chem. Res. 2: 344–352.
16. KERR, J. 1970. Relaxation studies on vicinal water. Ph.D. dissertation. University of Miami. Coral Gables, Florida.
17. LING, G. N. 1965. The physical state of water in living cell and model systems. Ann. N.Y. Acad. Sci. 125: 401–417.
18. PESCHEL, G. & K. H. Adlfinger. 1971. Thermodynamic investigations of thin liquid layers between solid surfaces. Z. Naturforsch. 26a: 707–715.
19. PRIVALOV, P. L. & O. B. Ptitsyn. 1969a. Determination of stability of the DNA helix in an aqueous medium. Biopolymers 8: 559–571.
20. PRIVALOV, P. L. & Y. I. TIKTOPULO. 1969b. Investigation of the thermal trans-conformation of procollagen. II. Sub-denaturing heat absorption in a solution of procollagen in the presence of salts. Biophys. 14: 16–25.
21. PRIVALOV, P. L., Y. I. TIKTOPULO & I. N. SERDYUK. 1969c. Viscosity of solutions of procollagen for low gradients of flow velocity. Biofizika 14: 1022–1031.
22. PRIVALOV, P. L. & Y. I. TIKTOPULO. 1970. Thermal conformational transformation of tropocollagen. I. calorimetric study. Biopolymers 9: 127–139.
23. SHEPHERD, J. C. W. & E. H. GRANT. 1968a. Dielectric properties of amino acid solutions. Proc. Roy. Soc. A307: 335–344.
24. SHEPHERD, J. C. W. & E. H. GRANT. 1968b. Dielectric properties of amino acid solutions. II. Dielectric dispersion in aqueous proline and hydroxyproline solutions. Proc. Roy. Soc. A307: 345–357.
25. UBBELOHDE, A. R. 1971. Melting and crystal structure. Proc. XIV Solvay Conf. : 111–147. Brussels, Belgium. May 1969.

DISCUSSION

DR. G. N. LING: I am very happy to hear your observation that a 0.1 μ separation of two surfaces seems to mark a major point of transition in the water molecules held between them, because we have come to the same conclusion. In your presentation, however, you expressed the opinion that no matter what kinds of surfaces these are, so long as you have an interface, you will have this water.

My second question is, why don't you think that the water in the living cell is in the multilayer state? Have you not contradicted what you just said about the effect of interfaces on water structure?

While I was reviewing the literature on the hydration of proteins, I found clear evidence that, as a rule, globular proteins hydrate to a smaller extent than fibrous proteins (Ling, G. N. 1972. *In* Water and Aqueous Solutions. R. A. Horne, Ed.: 663. John Wiley & Sons, Inc., New York, New York.) For this reason I think it could be misleading to reach conclusions on the physical state of water in living cells on the basis of knowledge derived from protein solutions in which the protein mostly exists in the globular state.

DR. DROST-HANSEN: I think that we are dealing to a large extent with a matter of semantics. What I understand you to say, both here and in your publications, is that when there are ionic sites on the proteins, these will induce structuring of the water molecules around them. These surely must be very high energy interactions, of the order of tens of kcal per g mol.

As I was careful to point out, I see very weak interactions as giving rise to some phenomena. These could not be dependent upon ion-water interactions, at least not of a long-range nature. Now surely, on the short range of one, two, or three molecular diameters, the water near an ionic surface is different from that near a nonpolar surface. What I am attempting to stress is that superimposed on this type of high-energy interaction are some peculiarities that genuinely seem to be independent of the specific chemical nature of the material to which the water is adjacent. I don't really think that there need be a conflict between your view and my view in this case. It is partially a matter of semantics, and perhaps it is also a matter of looking at two different phenomena.

DR. R. DAMADIAN: The question you have discussed with Dr. Ling is essentially whether water inside the cell is ordered because it is along ionic surfaces or along nonionic surfaces. This is slightly apart from the issue we are most curious about, namely, what is the actual situation for the state of water inside the cell?

For one cell at least (*E. coli*), I think we can take a reasonable guess. In this cell, we have comprehensively compiled all of the charges that make it up and computed the internal molality of the cellular charge. The intracellular solution is a 1 molal solution. Since water has a molality of 55, this means that the simple distribution of all the water molecules amongst all of the charge groups gives 55 molecules of water per charge group. From simple geometric calculation, this results in an average hydration atmosphere 2½ water molecule diameters thick for each of the charges.

I think everyone would agree that 2½ water molecular diameters are well within the polarizing range of an ionic field.

DR. DROST-HANSEN: George Safford, at Union Carbide, has used neutron inelastic scattering to study the structure of some aqueous solutions,

I think as high as 15 M potassium thiocyanate, and certainly of many of the alkali halides, up to several m. Safford finds that there are lines remaining in the solutions which are identical with those that occur in pure water. How this comes about I certainly don't understand. I would agree with you that if there is 1 ion to 50 water molecules one would imagine that most of the water molecules would be under the influence of the ionic fields. Safford's finding suggests that part of the water somehow or other may "remember" its original structure, at least under certain circumstances. As I mentioned before, the kinks appear regardless of whether you look at 0.5 M sodium chloride near an interface, or at pure water near an interface. Thus something is independent of the charges present—not completely independent, but essentially independent. This is all I can say. It does seem that we have a dilemma, and I cannot offer any solution.

DR. DAMADIAN: Let me make one refinement. What we have done is simply to distribute cell water among all the charges present. This does not take into account the solvation of any of the nonionic compounds. As a result, it is likely that the average hydration of a cellular ion is substantially less than 55 molecules per ion.

I would like to ask another question that is both important and short. What of the most famous of water's temperature anomalies, the anomaly at 4° C? Does it represent a "kink" by your definition? Does it conform to the general pattern of the anomalies you have described, even though it is out of phase with respect to the 15° C periodicity of the higher temperature, such as reversal of cation selectivity, cessation of growth, and so on, when there is no obvious reason for this abrupt change and when the bacteria remain frozen?

DR. DROST-HANSEN: I really don't know very much about the 4° C maximum. It is probably *not* one of the types of higher-order phase transition I have been dealing with in this paper. I do know that there are some strange things going on; for example, the rate of positron annihilation in water shows a noticeable jump at 4° C. God only knows why. I am sorry that in general I cannot address myself to that.

DR. BENDER (*Fairleigh Dickinson University, Rutherford, New Jersey*): Here you developed a potential of 30 V, and this was by virtue of the ionic separation of ions of opposite charge. I would expect that a strong interaction would do this, rather than a weak interaction.

DR. DROST-HANSEN: This would be the natural thing to expect, but it is apparent in the answer that this is not so. First of all, I must say that Workman deduced that the potential corresponds to a fantastic amount of energy. That would appear to be a strong interaction; but not if you consider the number of water molecules over which this is distributed, or which contribute to this. There is only a very much smaller amount of energy per water molecule if the cooperating units are of the order of 10^5 water molecules.

Unfortunately, or fortunately, as the case may be, Workman was wrong, because the ion is not taken in against a potential of 30 V *right* at the interface. Instead the potential is distributed over microns, or even millimeters, so that the gradient is much, much smaller. But fortunately Workman did not know this, so he went ahead and did the electron microscopy; because of this, we now know about the microstructure of ice crystals. Of course, 30 eV is a fantastic amount of energy, but again this is distributed over 10^5 water mole-

cules. There exists a real problem, at least in living cells, namely that apparently only very small energy differences are operating. How can you simultaneously maintain states that differ by only 0.1 kT separately? I don't know the answer. However, it is possible that the new approach by Glansdorff and Prigogine may give the answer for living systems. We are here so far away from equilibrium that the system, even on a macroscopic scale, may reorganize itself into local domains of order. I am suggesting that one should consider the water in the cells as one of the "elements" that may be responsible for structuring on a "macroscopic" scale (that is a poor choice of words). The nonequilibrium organization can apparently be "very macroscopic," as is seen in the currents in oceanic circulation. Here one is dealing with a very extensive system far from equilibrium, yet local "domains," hundreds of miles wide, occur as a means of achieving local "stability" and facilitating the dissipative processes.

EDWARD FINCH (*Bethesda, Maryland*): I would like to discuss an observation I made recently, which I believe has some direct bearing on this whole area of discussion. I occasionally do electron spin studies on biological samples and other aqueous solution samples; and with ESR techniques one is looking for absorptions that result from the interactions of a microwave magnetic field (in my case it is 10 GHz). Hence one wants to place a sample in the microwave cavity in such a way that it is out of the electric component of the electromagnetic field, and into the magnetic component. To do this scientists have developed what they call aqueous solution cells, which are normally made of quartz; they are flat cells less than 0.5 mm thick. Normally, if it is a rectangular cavity, they place this so that it is in the h plane, not in the electric field. All this works very well; losses from dielectric problems are minimized, and one gets nice results.

Recently I happened to rotate my sample cell 90° so that it was oriented into the electric field; now any position between 0° and 90° is bad, but once I reached 90° everything was restored. The dielectric losses were minimal. This was rather a unique observation, since evidently no one had ever tried it before. I have talked to the manufacturers of my spectrometer, and we have come to the conclusion that the dielectric properties of the water in that cell apparently differ. In other words, the dipoles are not free to orient in certain directions; there is some sort of wall effect. This would not mean that it would all be affected, but the electric dipoles must be affected. The water dipole is not free to rotate in the direction that would cause dielectric absorption when the sample is oriented perpendicular to the electric field of the microwave source.

DR. PHILIPPA WIGGINS (*Auckland, New Zealand*): I have been interested in other properties of surface water, and I found that the solubility of ions in water does change according to the chemical state of the surface. In a silica gel (in the purely hydroxyl form, in which it has a strong hydrogen bonding) ions such as sodium, hydrogen, lithium, calcium, and magnesium, the classical water structure ions, have less solubility in this highly structured water than in the bulk phase with which they are in equilibrium; but ions such as potassium, rubidium, cesium, chloride, sulphate, and nitrate, the classical water-structure-breaking ions, have higher solubility.

Now, if you destroy the hydrogen-bonding capacity of that surface by increasing the pH so that all the hydroxyl groups ionize, this selectivity is lost and the various ions are equally soluble in the two kinds of water.

DR. DROST-HANSEN: That is hardly surprising, perhaps, since the types of interaction that we talked about must occur between the dipolar and ionizable hydroxyl groups on the surface (in fixed locations) and the water adjacent to it. But I don't think that this is the type of water that I was talking about. In other words, here again the water that is directly influenced by a surface hydroxyl group is bound much more tightly than the interactions between neighboring water molecules in that domain. It is the additional water that I am interested in, and which I feel is responsible for exhibiting the thermal anomalies.

Somehow or other the two must connect, and in a sense we have here a dialogue not unlike the one with Professor Ling. There must certainly be some connection between the two; I just cannot delineate it here in any detail.