

"Thermal Anomalies in Membrane Properties"

by

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I. Background

It is generally recognized that water assumes a highly anomalous position among liquids because of its structural properties. Furthermore, these properties play an important role in many biologic phenomena.

In the present paper we shall be concerned with the existence of "anomalies within the already anomalous properties" of water: in particular, anomalous temperature dependencies. Plotting a number of properties of water as a function of temperature has revealed the existence of more or less abrupt changes in thermodynamic and transport parameters at discrete temperatures. As an example, Figure 1 shows the transverse proton spin relaxation time as a function of the inverse absolute temperature as determined by Brown (1958). One notices a remarkable change in slope in the vicinity of 60°C. The range of data shown in this illustration was from 40 to 100°C. Brown, however, noted that anomalies appeared also in the vicinity of 42 and 18°C. Another example is shown in Figure 2. Here the mean activity coefficient for sodium chloride in water is plotted as a function of temperature. The illustration taken directly from Harned and Owen's monograph "The Physical Chemistry of Electrolytic Solutions", (1958). In Figure 3 we have deleted the original curves and instead drawn curves which we believe present a more reasonable fit to the actual data. One notes here the divergence of the points below 40 and above 60°. Furthermore, it appears that the data above 60° fall on very nearly straight lines. Finally, there is evidence of reversed curvature in the region from 30 to 40°.

II. Higher Order Phase Transitions

From a review of the properties of a vast number of aqueous systems it has been possible to make a number of generalizations. These have been discussed separately in previous publications by the senior author (1965b, c; see also Lavergne & Drost-Hansen, 1956).

1. At least four thermal anomalies occur between the melting and boiling points of water, mainly near 15, 30, 45 and 60°C.
2. The thermal anomalies - which for want of a better name have become known as "kinks" - occur both in the properties of pure water and aqueous solutions. For this reason, it is believed that the kinks owe their existence to some phenomena associated with the structure of water. The existence of kinks in aqueous solutions (and other aqueous systems) is believed due to the persistence of particular structural features of water itself, even in the presence of electrolytes and non-electrolytes at high concentrations.
3. The temperatures at which the kinks occur are not sensitive to the nature or concentration of the solutes. This is particularly important in the application of these notions to biologic systems. Only strong acids in moderate to high concentrations change the temperatures at which the kinks occur. In solutions of strong acids, the kinks are still found, but the temperatures are notably changed from the values for pure water.
4. The kinks are present in both equilibrium properties and transport phenomena.
5. The kinks appear in both surface and interfacial phenomena. However, the temperature at which the kinks occurs in many surface phenomena may be slightly different from the temperatures where the kinks are found in bulk systems.

The anomalies are very likely manifestations of higher order phase transitions in the water. At this time the structure of water has eluded final determination. Of the several models of water currently discussed we believe a cluster model (such as the Nemethy-Scheraga (1962) model) and/or a cage model (such as suggested by Pauling (1959) or Frank and Quist, 1961) is most likely to be correct. In terms of these models the kinks then represent more-or-less sudden transitions from one stable configuration (cluster or cage) to another such structured species.

III. Application to Biological Systems

From our studies on water near interfaces we believe that the kinks are particularly prominent in such systems. This is significant for biological systems where much of the water is found intimately associated with various interfaces. Because of the interfacial aspects we became interested in the effects on membrane phenomena of the structural transitions of water. However, before discussing membrane phenomena we shall show a number of examples of the role of structural transitions in water on a number of systems of general biologic interest.

Figure 4 shows the temperature dependence of the viscosity of protoplasm from Cumingia eggs. These data by Heilbrunn (1924) clearly show a notable maximum in the vicinity of 15° or 16°C with a minimum near 2° and near 30 or 31°C. While we do not at this moment understand the sudden increase in relative viscosity below 2°C, it appears likely that the sharp maximum at 15° and the pronounced minimum near 30° are associated with the underlying water structure changes.

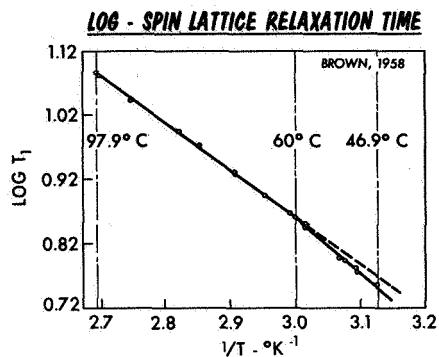


Figure 1.

Transverse relaxation time for protons in water as a function of temperature. Data by Brown.

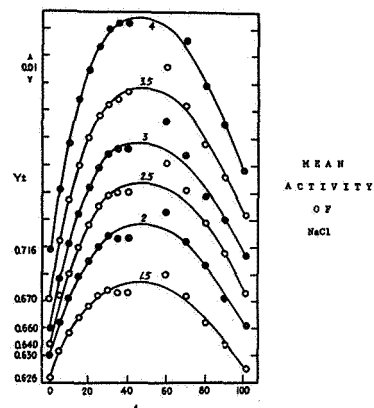


Figure 2.

Mean activity coefficients for sodium chloride in water as a function of temperature. This illustration is taken directly from Harned & Owen's monograph: "The Physical Chemistry of Electrolytic Solutions." (By permission)

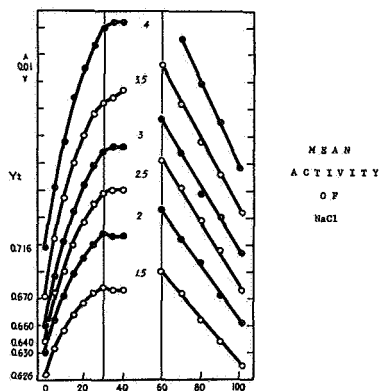


Figure 3.

Mean activity coefficients for sodium chloride in water as a function of temperature. Same data point as in Figure 2, but curves redrawn by senior author.

PROTOPLASMIC VISCOSITY, CUMINGIA EGGS

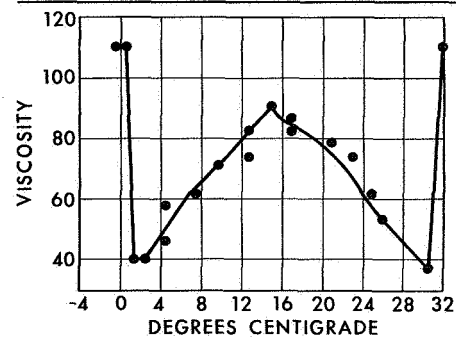


Figure 4.

Viscosity of protoplasm for Cumingia eggs.

Another example of the anomalies near 30 and 45° is shown Figure 5, taken from a paper by Rao and Engelberg (1965). This illustration shows the maximum mitotic index as a function of temperature. Apparently, near the temperatures of the structural transitions - that is near 30 and 45° - a significantly larger number of cells are entering the mitotic stage than are leaving it. It is undoubtedly important in this context that water loss accompanies the mitotic stage. Lower mitotic index and therefore higher rate of cell division occurs near 37° as anticipated by Oppenheimer and Drost-Hansen (1960).

Cell division and cell growth in general appear to be exceedingly sensitive probes of the changes in the water structure. Growth is generally retarded in the vicinity of the temperatures of the structural transitions (the kinks) while optimum growth prevails roughly midway between consecutive kinks. Although it is not possible at present to relate directly growth phenomena to the associated membrane phenomena without oversimplifying, we show in the following a number of examples of growth as a function of temperature to emphasize the above-mentioned temperature sensitivity. Thus Figure 6 shows the rate of growth of a mutant of *Neurospora crassa* in media of insufficient riboflavin supplement (Mitchell and Houlahan, 1946). The existence of multiple growth optima is clearly shown. The optima for growth occur near 25 and 37° and the minima for growth occur near 15, 30 and 45° - the temperatures of the kinks.

Another example of multiple growth optima is shown in Figure 7. This illustration, taken from a recent paper by Davey, Miller and Nelson (1966) shows two growth optima near 26° and 34°C, again with a distinct minimum near 29°C.

V. Membranes

We now address ourselves to the problem of temperature effects on membranes. The senior author (1965a) has recently presented a detailed study of the nature of aqueous interfaces in general. The structural changes in water appear reflected (and frequently greatly enhanced) in interfacial phenomena of all types. For this reason, we became interested in membranes as the interfacial phenomena must here play a dominant role. Thus, our current studies are aimed at determining the effects of temperature on the properties of a wide range of membranes, from the simplest types of physico-chemical membranes (such as liquid membranes) to complex, naturally-occurring biologic membranes.

A review of the literature has revealed a notable lack of detailed information about temperature effects on membrane phenomena. One of the few studies which has been concerned with temperature effects on an exceedingly simple membrane is that by Schulman et al. (Rosano, Doby & Schulman 1961). The rate of transfer of salt and water across a nonaqueous liquid phase was studied at a number of temperatures. The liquid used as a "membrane" in this study was n-butanol, separating two aqueous phases of different concentrations. Figure 8 shows the experimental set-up used by Schulman and co-workers. The butanol separates the two aqueous phases; transfer of ions between the phases occurs via the butanol. All phases are stirred to avoid concentration gradients in the bulk phases; the passage across the interfaces is the rate-determining step. Figure 9 shows the results obtained by these authors for the rate of transport of sodium chloride across the butanol membrane. The ordinate is the logarithm of the rate of transfer (as measured by the rate of change in the emf); the abscissa is the reciprocal, absolute temperature. One notices the abrupt change (in fact, overt discontinuity) in the data in the interval between 30 and 39°. It is unfortunate that no data were obtained in this temperature interval. We are presently repeating measurements of this type and hope to obtain for more closely spaced data. It is interesting, though, that the results obtained by Schulman and co-workers for the rate of transfer of potassium chloride very closely resemble the data obtained for the transfer of sodium chloride: the potassium chloride data are shown in Figure 10 and again one notices the marked discontinuity in the data between 30 and 39°. Furthermore, it is observed that the slopes for both the KCl and NaCl data are almost identical in the range from 39 to 45° and again, are almost identical for the two salts below 30°. We believe this lends credence to the fact that a notable structural transition has occurred around 30°.

The membrane studied by Schulman and co-workers is obviously of limited significance in connection with biologic systems. Butanol only poorly mimics the behavior of a lipid membrane. However, more recently Thompson (1964) has studied the properties of a bimolecular phospholipid membrane. Thompson measured the resistance across this membrane at closely-spaced temperature intervals and Figure 11 shows his results. One notices in this illustration the large maximum near 23° and the marked transition in the vicinity 29-30°C. While it is relatively simple to appreciate the general decrease in resistivity as the temperature increases in the region from 23° and 29° and from 30° to 36°, it is considerably more difficult to understand the mechanism by which the apparent resistance of this membrane increases between 20 and 23°. However, the increase in resistance between 29 and 30° is undoubtedly a reflection of a changed water structure in or adjacent to this bimolecular membrane. Thompson feels there may be a continuous aqueous phase across the membrane and that the anomaly may be due a higher-order phase transition.

Finally, we mention an example of a study of a natural membrane, namely the plasma membrane of *Valonia macrophysa*. Figure 12 shows the results obtained by Blinks (1942) who measured the potential difference across the membrane at a number of temperatures. It is apparent that the values from 15 to 30° are relatively constant, independent of the temperature while the emf increases both below 15 and

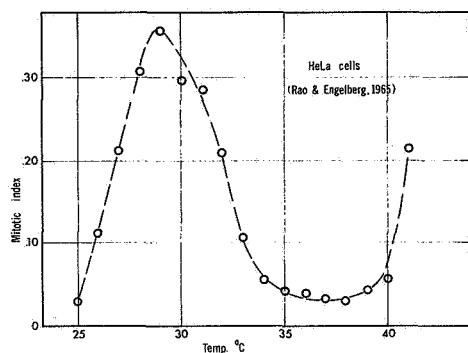


Figure 5.
Mitotic accumulation in HeLa cells. Data by Rao and Engelberg (1965).

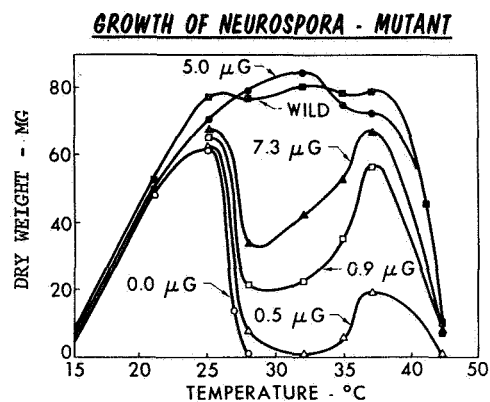


Figure 6.
Growth of a mutant of *Neurospora crassa*. Data by Mitchell and Houlahan (1946).

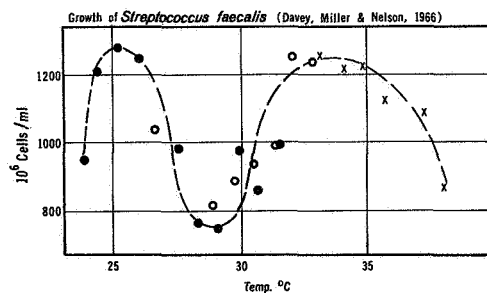


Figure 7.
Growth of *Streptococcus faecalis*. Composite graph of three independent runs. Data by Davy, Miller and Nelson (1966).

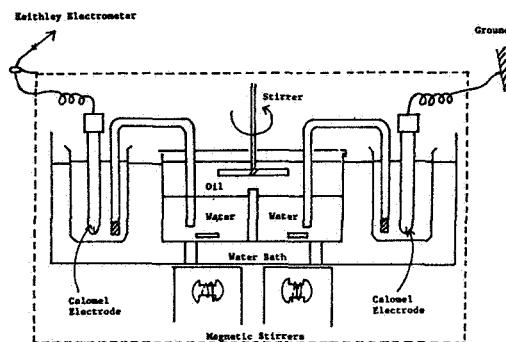


Figure 8.
Schulman's transport cell for liquid membrane studies. Rosano, Duby & Schulman (1961). (By permission, J. Phys. Chem.)

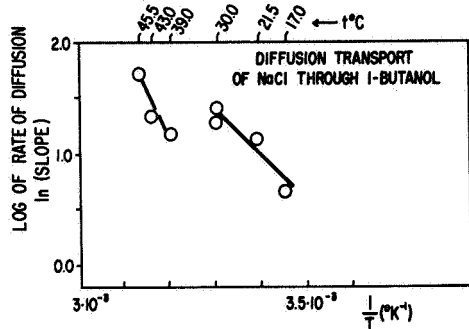


Figure 9.

Log rate of diffusion (= log rate of change of potential) of sodium chloride across a "butanol membrane" as a function of reciprocal, absolute temperature. See text. Data points: Schulman et al. Curve redrawn by the senior author.

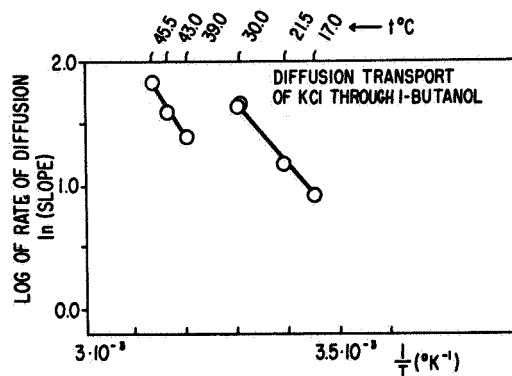


Figure 10.

Log rate of diffusion (= log rate of change of potential) of potassium chloride across a "butanol membrane" as a function of reciprocal, absolute temperature. See text. Data points: Schulman et al. Curve redrawn by the senior author.

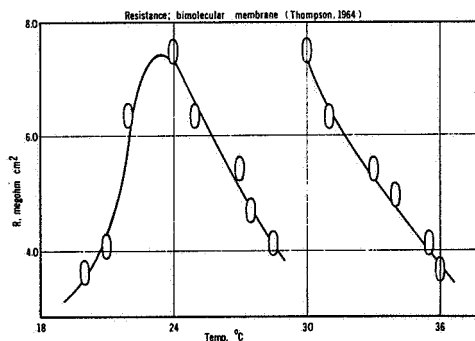


Figure 11.

Temperature dependence of phospholipid, bi-molecular membrane. Data by Thompson (1964).

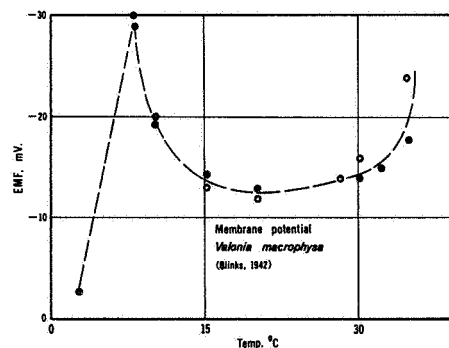


Figure 12.

Potential across plasma membrane, *Valonia macrophyssa*. Open circles: increasing temperature. Filled circles: decreasing temperature. Blinks (1942).

above 30°C. As was the case with the results obtained by Thompson, it is not possible at this time to propose a detailed definite mechanism responsible for the observed temperature dependence of the potentials. However, the temperature dependence does suggest that the structural aspects of water discussed above may play a role in determining the properties of this membrane.

With these examples, we wish merely to draw attention to the fact that the underlying structural changes in water may likely affect membrane phenomena (as well as many other biologically important parameters) and urge that the structural changes in water be considered in further studies on membranes.

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