

THE EFFECTS ON BIOLOGIC SYSTEMS OF HIGHER-ORDER PHASE TRANSITIONS IN WATER*

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The purpose of this conference is to discuss "Forms of Water in Biologic Systems." This undertaking is very ambitious and most likely our achievements at the present time will be limited. The principal difficulty before us is simply that we do not as yet possess a picture of the structure of water or aqueous solutions that has any degree of finality about it. Hence, it is indeed somewhat presumptuous for us to try to discuss various forms of water in biologic systems as long as we are without any appreciable understanding or agreement about the structure of bulk water or aqueous solutions. We might well add to this regrettable state of affairs our lack of knowledge of the structure of water near such simple interfaces as the air-water interface, water-immiscible liquid interface, or the structure of water near any simple solid-water interface, such as may exist near the surface of a mineral grain.

Theories of Water Structure

A few years ago, Henry Frank (1963) gave a brief but eloquent survey of the current status with respect to theories of liquid water and more recently Kavanau (1964) has reviewed the current theories of water structure in some detail. From these reviews, one sees the great range of physical models that have been invoked over the past 30 years to account for the properties of water. These theories range from the "purists'", "average" models of liquids — due primarily to physicists and other strictly "theoretical" liquid structure researchers — to the models that Henry Frank has termed the "mixture models." The mixture models embrace a variety of models from simple water-ice mixtures to water polymers and water clusters. These include Euckens polymer model (consisting of small polymeric species of water), the more recent cluster and cage models, and the "super clusters" (the older cybotactic groups of Stewart, Frenkel and Nomoto). The current status of water structure research certainly lacks finality. Because of the present state of knowledge, any discussion of water in biologic systems must definitely be limited. Without knowing the structure of water, how can we predict or understand the influence that the multitude of solutes in biologic systems have on the structure of water, or

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how can we describe the water near the all-dominating interfaces of biologic systems? The unsettled question of the existence or nonexistence of long-range ordering in water adjacent to a solid surface, for instance, must surely be decided before we can hope to understand in detail the behavior of water in membrane systems.

Thermal Anomalies

The present paper is a summary of 10 years' thinking by the author and his coworkers with regard to water in biologic systems. The major shortcoming — and at the same time perhaps the major advantage — of our approach is that we do not build on any specific model for the structure of water. Rather, our speculations are based on the observation, sporadically reported in the literature, of the existence of thermal anomalies in the properties of water and aqueous solutions. Over the past decade, we have studied the properties of water and a large number of aqueous solutions and have become convinced that the properties of liquid water do exhibit anomalies at a number of different temperatures and that these anomalies reflect more or less abrupt structural changes in the water. Such anomalies, which for want of a better name have become known as "kinks," have been suggested previously by several other authors. For instance, Dorsey (1940) in his monograph points out that the measurements of magnetic susceptibility by Wills and Boecker and by Seely seem to indicate anomalies in the vicinity of 35 to 55°C., and Dorsey also points out that Tammann expected the existence of an anomaly near 50°C. Likewise, in 1935 Magat (1935) proposed that anomalies exist in the properties of water near 40 to 45°C. based on measurements of solubility, viscosity, compressibility, and specific heat. Antonoff (1950) and Forslind (1952) have also studied in some detail the more or less abrupt transitions in the properties of water, and other authors have, over the years, sporadically mentioned the existence of kinks. Forslind has specifically suggested that anomalies exist at 12, 35 and 55°C.; however, the evidence presented by Forslind was not very extensive. Ives and coworkers (1963) have discussed the existence of anomalies in the properties of water and aqueous solutions and postulated an anomaly near 30-35°C. Attention is called in particular to Feates and Ives' (1956) study of the dissociation of cyanoacetic acid, and Franks and Ives' (1960) study of interfacial phenomena.

The kinks are evident in the data from many different types of aqueous systems, and the present author has come to believe that at least four kinks occur between the freezing and the boiling point of water, namely near 15, 30, 45 and 60°C.

Evidence

It is not possible to detail here all the evidence available to show the reality of the anomalies in the properties of water and aqueous solutions;

a general review of the evidence will be presented elsewhere (Drost-Hansen, 1965). In this section we show only a small group of examples to indicate the diversity of conditions and phenomena in which the anomalies are observed in both pure water and aqueous solutions. In *General Observations* is a brief summary of the major generalizations and conclusions that can be drawn from an examination of all the available data. Finally, in sections *Biologic Implications* and *Discussion* the evidence for the role played in biologic systems by the anomalies observed in water and aqueous solutions is discussed.

The kinks are manifested in both the properties of pure water and of aqueous solutions. It is instructive first to consider the evidence available from measurements on pure water. One difficulty frequently encountered in searching for the anomalies is the lack of measurements of any one parameter at sufficiently closely spaced temperature intervals. There are, however, a few cases in which parameters have been measured at enough temperatures (over a relatively narrow temperature range) to permit a statistical analysis of the resulting data. One such example is the density of water as determined by Chappuis at the International Bureau of Weights and Measures (1907). We have previously analyzed and published these data (Lavergne & Drost-Hansen, 1956) and we shall only briefly summarize the results here. The data obtained by Chappuis extend from near zero to 41°C. Rather than work with the density itself, the reciprocal of the square root of the density was used, in other words, the square root of specific volume. Plotted against temperature this has, to a rough approximation, the form of two intersecting, straight lines, the intercept being near 4°C. Considering only the data from about 5 to 41°C., it appears as if there are three distinct, separate curve segments, namely, one from 5 to 15°C., one from 15 to 30°C., and one from 30 to 41°C. By the method of least-squares best-fit, second order polynomials were fitted to each of the three curve segments and separate curves were fitted for the two combined intervals from 5 to 30°C. and from 15 to 41°C. By an F-analysis, it was possible to show, taking properly into account the added degrees of freedom when using separate curve segments for each interval, that the fitting of two curves over two intervals gave significantly better fit than one curve through any combined interval. Hence, it was concluded that there appears to be anomalies near 15 to 30°C.

We have analyzed in a similar fashion the dielectric constant data obtained by Devoto (as quoted by Dorsey, 1940). In this case, the data extend to slightly above 30°; hence, the analysis was made to test only for an anomaly in the vicinity of 15°C. and it was again found highly significant statistically that two separate curve segments give a better fit than one curve through the combined interval from zero to 30°C. An examination of the more recent dielectric data on water by Malmberg and Maryott

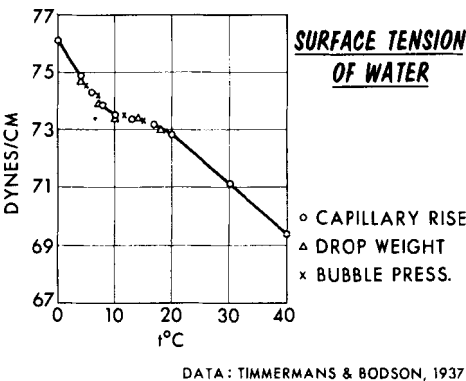


FIGURE 1. Surface tension of water determined by Timmermans & Bodson (1937).

(1956) shows a minimum in the relative temperature coefficient ($1/\epsilon$) ($d\epsilon/dt$) near 60-65°C. and very possibly an inflection point in this quantity near 30-35°C. An anomaly in the specific heat of water has been proposed earlier by Magat, Dorsey and Feates and Ives (1956). Further evidence for anomalies in the properties of pure water is found in particular in the temperature dependence of many surface and interfacial phenomena. Thus, Franks and Ives (1960) have noted an inflection point near 34°C. in the interfacial tension between water and n-hexane. The present author (1964) has recently examined some other surface and interfacial properties of water and aqueous solutions and found considerable evidence for the existence of the kinks in such systems as well as evidence for structural discreteness near interfaces. As an example, FIGURE 1 shows the surface tension of

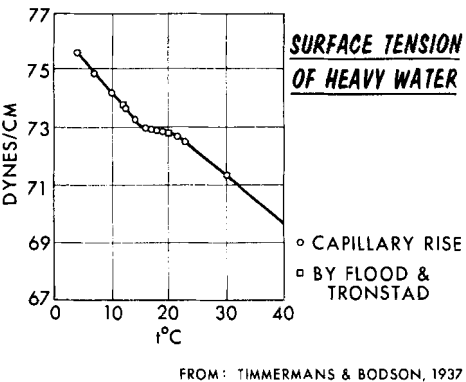


FIGURE 2. Surface tension of heavy water. Timmermans & Bodson (1937).

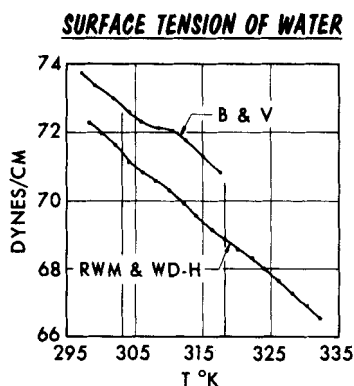


FIGURE 3. Surface tension of water. Data by Bordi & Vannel and by Drost-Hansen and Myers.

water as determined by Timmermans and Bodson (1937) in the middle thirties. FIGURE 2 shows the data for heavy water obtained by the same authors. It is remarkable that such a pronounced inflection point should have been observed by these authors but gone unrecognized by other investigators of the surface tension of water. A careful inspection shows, however, that inflection points are indeed apparent in practically all other measurements of the surface tension of water where sufficiently closely spaced data have been obtained. Thus, in data as old as those obtained by Brunner (1847) and Wolf (1857) one can recognize the presence of an inflection point near 15°C . Other authors have suggested anomalies in the surface tension of water at other temperatures and FIGURE 3 shows the surface tension of water as obtained respectively by Bordi and Vannel (1962)

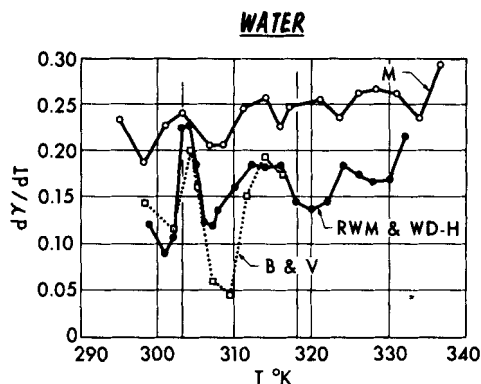


FIGURE 4. Derivative of surface tension with respect to temperature. Based on data by Moser, Bordi & Vannel, and Drost-Hansen and Myers.

in Italy and by the present author and Myers (1963). Again, inflection points are observed near 30 to 33°C. and 45°C. (present author's data). Finally, FIGURE 4 shows the "entropy of surface formation" as reported by Drost-Hansen (1965). The three curves depict the numerically differentiated surface tension data obtained respectively by Moser (in 1927), Bordi and Vannel, and Drost-Hansen and Myers. It is interesting that agreement exists to the extent shown in FIGURE 4 with regard to the temperature derivative of the surface tension. Although it is not permissible to identify directly this quantity with the entropy of surface formation, the data do indicate an abrupt change of ordering near 30°C. For a more detailed discussion, the reader is referred to the article by the present author.

Anomalies in the viscosity of water and aqueous solutions as a function of temperature have been noted by several authors, for instance, Forslind (1952), Magat (1935), Neill and Drost-Hansen (1955), de Carvalho (1944), Qurashi and Ahsanullah (1961) and Othmer and Thakar (1953). In a study of the viscosity and diffusion in solutions, Othmer and Thakar state that "where water is the solvent, the lines show a definite break at about 30°C. An exactly similar break was found to occur near the same temperature when viscosity of water was plotted in a similar way against a vapor pressure of water."¹⁰ They also state with respect to aqueous solutions: "The temperature at which the changes in internal structure of water occur may vary somewhat with the dissolution therein of the solute; hence, the breaks in the line may be at somewhat different temperatures in the general range of 30°C."

Nuclear magnetic resonance studies have also suggested the existence of changes in the structure of water according to several authors. Brown (1958) has suggested that anomalies exist in the transverse relaxation times for water in the vicinity of 18, 42, and 60°C. FIGURE 5 shows a plot

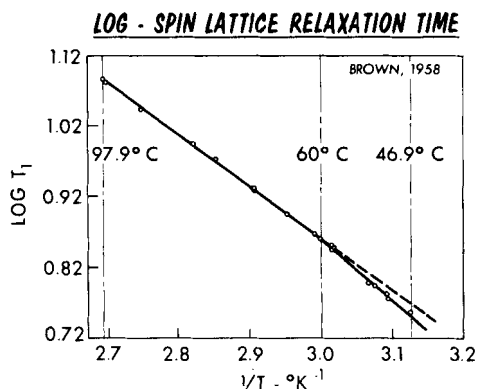


FIGURE 5. Transverse relaxation time for protons in water. Data by Brown.

of some of Brown's data: the ordinate is log transverse relaxation time while the abscissa is the reciprocal absolute temperature. The kink near 60°C. is fairly pronounced. An anomaly in the NMR data is also suggested by Simpson and Carr (1958) in the vicinity of 40-45°C. on basis of their NMR studies.

Spectroscopic evidence for anomalies in water has been suggested by Magat (1937) near 40°C. Other evidence for the existence of anomalies in the properties of pure water may be obtained from the data by Pinkerton on the ultrasonic amplitude absorption coefficient (1947). FIGURE 6 shows

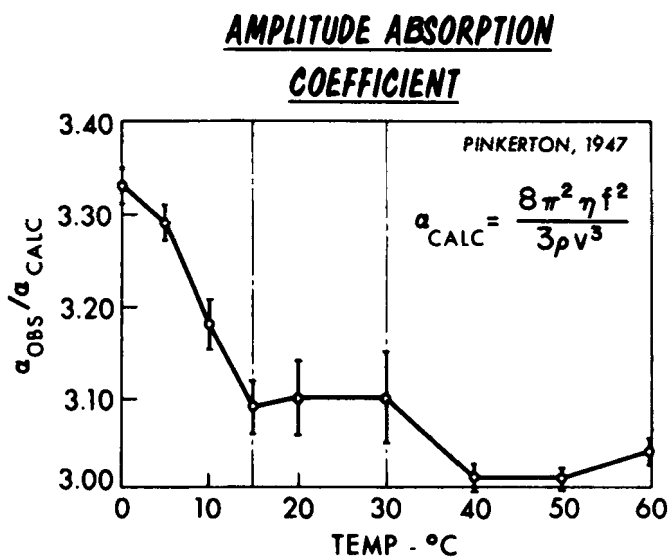


FIGURE 6. Excess ultrasonic sound absorption coefficient. Based on data by Pinkerton.

Pinkerton's data point and his estimated errors on the determinations. It should be noted that the limits of error suggested by Pinkerton appear to be unnecessarily large for the data points near 20 and 30°C., thus the apparent anomalies at 15 and near 30°C. seem very likely to be real. The thermal conductivity data obtained by Frontas'ev (1956) are also suggestive of an anomaly and Frontas'ev states: "It can be assumed that a fundamental modification in the structure of water takes place in the range of 30 to 40°C. which is reflected in the indicated anomaly in the thermal conductivity polytherm."

Much skepticism has been voiced with regard to the reality of the anomalies discussed above. One frequent criticism has been that it often appears that each investigator has his "choice" temperature or temperatures

for the anomaly (or anomalies). While unfortunate, this in itself can hardly condemn the notion that anomalies may indeed exist in water and considering the present author's contention that at least four anomalies exist, it is obvious that a considerable choice exists for anyone concentrating on a particular range of temperatures. While the temperatures of approximately 15, 30, 45, and 60°C. may appear unnecessarily "generalized" and "symmetrical," evidence will be presented elsewhere in favor of the transitions being at least in the vicinity of these temperatures.

"Kinks" in Properties of Aqueous Solutions

We shall briefly show a number of examples of anomalies in the properties of aqueous solutions. While the existence of the kinks is often difficult

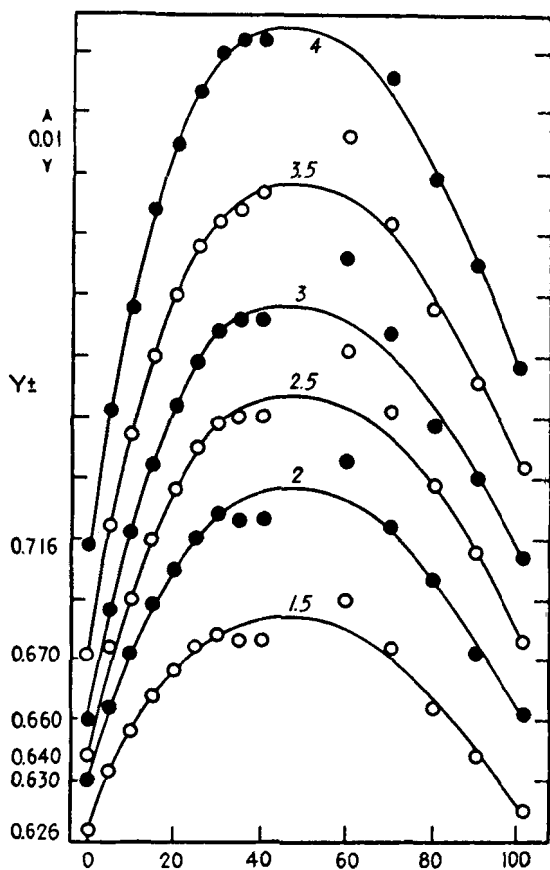


FIGURE 7. Mean activity coefficients for sodium chloride in water as a function of temperature. This illustration is taken directly from Harned & Owen's Monograph: "The physical chemistry of electrolytic solutions". (By permission).

to demonstrate unambiguously on any one particular set of data obtained on pure water, it is often easier to find more unique evidence for the anomalies in the properties of aqueous solutions. In particular, such areas as solubility, kinetic data for hydrolysis, and optical rotation of aqueous solutions seem frequently to exhibit the kinks particularly clearly.

FIGURE 7 shows the activity coefficients for sodium chloride in water as a function of temperature. This illustration is taken directly from Harned and Owen's monograph (1958); it shows the experimental values of the activity coefficient for sodium chloride in water and smoothed curves to fit the data. In FIGURE 8 we have shown the same points but omitted the smoothed curves; instead, we have drawn a series of curves which we be-

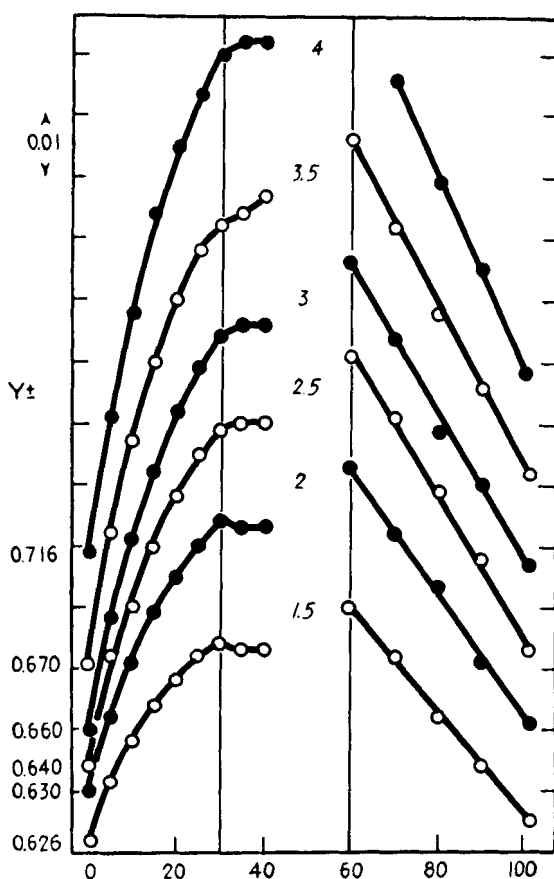


Figure 8. Mean activity coefficients for sodium chloride in water as a function of temperature. Same data point as in FIGURE 7 but curves redrawn by present author.

lieve represent far more realistically the temperature dependence of the activity coefficient. Unfortunately, there are no data between 40 and 60°C., also, the data below 40°C. were obtained from electromotive force measurements while the measurements from 60 to 100°C. were obtained from boiling point elevations. While it is unfortunate that different methods of observation were used for the different ranges studied, it seems significant that there are pronounced trends in the data. Thus, it is difficult to see how one can escape the conclusion that the activity coefficient to a first approximation decreases linearly with temperature in the region above 60°C. Secondly, in all cases there is a notable "gap" between the data at 40°C. and the data at 60°C. Furthermore, it seems that an actual reversal in curvatures may exist for the 1.5 and 2 molar solutions near 30°C. The diameter of the circles representing the measured values is 0.002 in γ_{\pm} , corresponding to, respectively, 0.15 mV or 0.003° in the boiling point elevation. Considering the accuracy and in particular the precision with which EMF's may ordinarily be measured, the deviations — even from the very optimistic, smooth curve of FIGURE 7 — seem highly significant. As pointed out above, the *trend* in the data is exceedingly difficult to ignore. Note also the amazingly high concentrations for which the anomalies seem to exist — up to three to four molar.

FIGURES 9 and 10 show the concentration independent term for the partial molal volume for potassium chloride and potassium iodide; the data

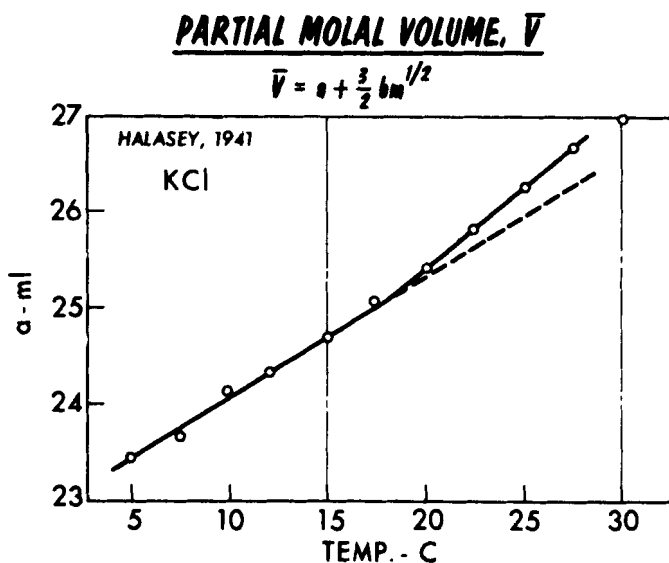


FIGURE 9. Concentration-independent term, a , in the equation for the partial molal volume of potassium chloride $v_1 = a + (3/2) \cdot b m^{1/2}$.

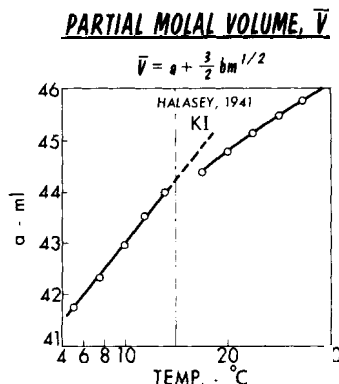


FIGURE 10. Concentration-independent term, a , in the equation for the partial molal volume of potassium iodide.

were obtained by Sister Halasey (1941). In the two systems shown, the kinks do not occur exactly at the temperature mentioned earlier, namely 15°C., but certainly in the vicinity of that temperature. It is of interest also to notice the opposite trends in changes of slope for the two salts in the vicinity of 15°C.

Solubility data often reflect the kinks particularly clearly, and Magat (1935), for instance, used the minimum in solubility of bromine in water near 30°C. as a suggestion of an anomaly in the vicinity of that temperature.

FIGURE 11 shows a recent example of solubility as determined by Klots and Benson (1963). These authors determined the solubility ratio between argon and nitrogen. The left side of FIGURE 11 shows the data and the

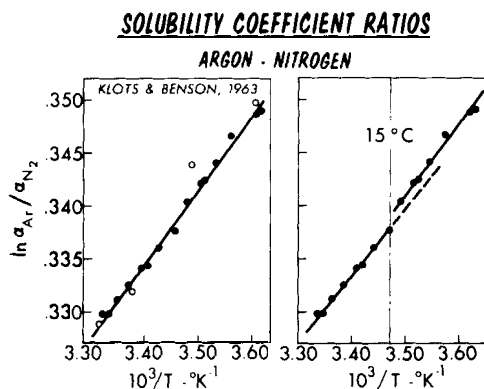


FIGURE 11. Solubility ratio between argon and nitrogen as determined by Klots and Benson.

smoothed curve originally suggested by Klots and Benson, obtained by a manometric method. The right side shows the same data but instead of one curve, two separate curve segments have been fitted to the data. As previously done for the density and dielectric constant data, we have made a statistical analysis of the solubility data. Again it was found highly significant that two separate curve segments give a better fit than one curve through the combined intervals. It is indeed interesting that the kinks show up so markedly in the present case where we are dealing with *ratio* of solubility of two quite similar gases, especially if one attempts to relate the solubility of these gases to their clathrate-forming tendencies. Both gases form Type I hydrates and both have almost the same Parachor (van der Heem, 1964), hence, it is particularly hard to find any reason here for specificity; it should be noted, however, that the dissociation pressure of the hydrates at 0°C. is only 95 atm. for argon but 160 atm. for nitrogen.

Many other solubility studies suggest the existence of the kinks. It is unfortunately quite rare to find the solubility of any particular solute in water studied at sufficiently closely spaced temperature intervals to permit one to look for all four kinks. However, the kinks are quite noticeable, for instance, in the solubility of lanthanum chloride (data from Seidell, 1940) as shown in FIGURE 12 where the kinks near 45 and 60°C. are quite distinct although apparently displaced a few degrees from the values for the temperatures for the kinks in pure water and dilute solutions. Note the high concentration for which the kinks seem to persist (the solid phase in equilibrium with the solution is the heptahydrate throughout the temperature range investigated). Anomalies are indeed often found for very high concentrations. Thus, when the usual plot of logarithm of the solubility versus $1/T$ is made, the data for the solubility of silver nitrate obtained by Campbell and Boyd (1943) also show quite distinct anomalies near 10 to

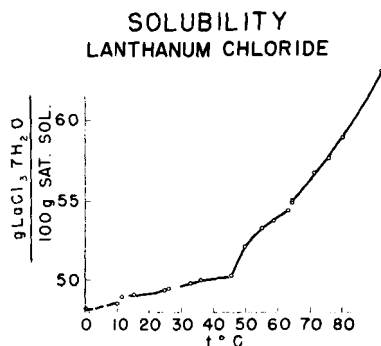


FIGURE 12. Solubility of lanthanum chloride heptahydrate versus temperature.

15°, 25 to 30° and in the vicinity of 60°C. Here the molar ratio of solute to solvent is approximately 1:4. Similarly, the solubility of substances like *r*- and *l*-mandelic acid also shows the kinks, again for extremely high molar ratios of solute to solvent. Among many other examples, we might mention the solubility of thiourea (Jänecke & Hoffman, 1932), thallium nitrate, thallium hydroxide, silver iodate, silver nitrite, barium sulfamate, ether and benzoic acid (Seidell, 1940).

General Observations

A large number of other examples could be cited as evidence for the reality of the anomalies in water and aqueous solutions; however, because of the limited time we refer to the forthcoming monograph which will present in greater detail the total evidence available. Before proceeding we summarize the main conclusions which we have drawn with respect to the thermal anomalies in water based on all the available evidence (see Drost-Hansen, Sept. 1963).

- A. The temperatures of the kinks are approximately 15, 30, 45 and 60°C. within $\pm 1^\circ - 2^\circ$ of these temperatures; this is to say that kinks may be centered, for instance, near 13° and 31°C. while the next kink may occur at 44°C. Hence, the glaring symmetry that would otherwise be implied is not necessarily real, and the enumeration of the 15°C. multiples may serve conveniently and only as a mnemotechnic device for remembering the temperatures of the transitions. The changes occur, as best as one can tell, over a fairly narrow temperature interval, about one to two degrees on either side of the "center" of the transition temperature. It is possible, and in fact likely, that more than four kinks exist. I believe the next kink would be found for temperatures in excess of 80°; however, we do not yet have enough data to propose any specific temperature. Very likely there is also a kink in the vicinity of 140-160°.
- B. The kinks occur in the properties of both pure water and aqueous solutions. For this reason, we believe that the kinks owe their existence to some phenomena associated with the structure of water and that the existence of the kinks in aqueous solutions is due to the persistence of particular structural features of pure water in the presence of solutes.
- C. The temperatures at which the kinks occur in aqueous solutions are rather insensitive to both the nature and the concentration of solutes. We have not yet sufficient material on hand to state the excursions to be expected from the pure water values for the temperatures of the kinks as a function of concentration or nature of the solute. However, generally the changes are probably within a few degrees for most solutes up to concentrations of the order of, say, two-five molar.

Only strong acids in moderate to high concentrations seem to change the temperatures of the kinks to any appreciable extent. The kinks, how-

ever, remain but the temperatures are noticeably changed from the values for pure water and dilute solutions.

D. The kinks are present in both equilibrium properties and in transport phenomena.

E. The kinks show up in surface and interfacial phenomena as well as in bulk properties. For surface phenomena the kinks may occur at slightly different temperatures than observed for bulk phenomena.

Biologic Implications: "Simple" Kinetic Phenomena

It has been claimed that there exist in water structural transitions that are manifested in the temperature dependence of many properties. We have also claimed that these transitions occur even in very concentrated aqueous solutions and have inferred that the occurrence of the transitions in such solutions must reflect the persistence of elements of structure that are unaffected by the presence of the solute. Hence, it is reasonable to propose that the water in biologic systems may undergo similar transitions.

Over the years, many authors have discussed the possible existence of more or less abrupt anomalous changes in biologic phenomena as a function of temperature. FIGURE 13 shows an example of such an alleged, abrupt

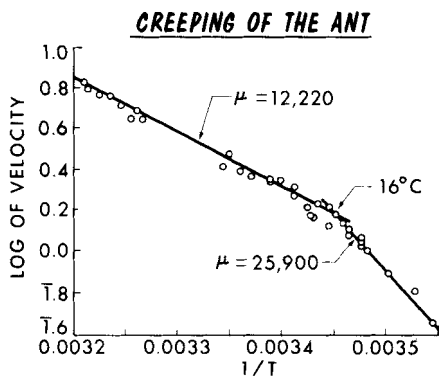


FIGURE 13. Rate of creeping of ant as a function of temperature.

change with temperature. This illustration shows the logarithm of the rate of creeping of the ant as a function of the reciprocal, absolute temperature. This illustration is redrawn from Johnson, Eyring and Polissar's book, *The Kinetic Basis of Molecular Biology* (1954); the data were obtained by Shapley and have been discussed by Crozier. Please note that the previous authors have kindly provided their estimate of the temperatures at which the two straight lines intersect. FIGURE 14, also redrawn from the book by Johnson, Eyring and Polissar, shows the temperature dependence

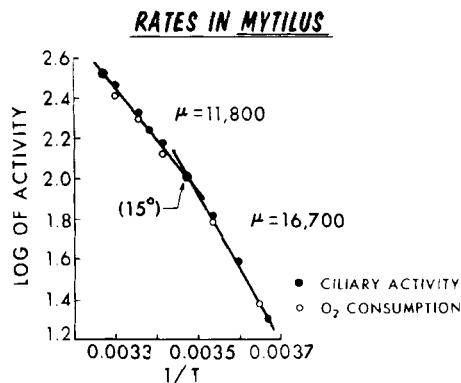


FIGURE 14. Rate of ciliary activity and oxygen consumption of the gill epithelium in the clam, *Mytilus*.

of two other phenomena, namely, ciliary activity of the clam *Mytilus*, which is shown as solid circles, and the oxygen consumption of the gill epithelium of the same clam, shown as open circles. These data are by Gray, worked up by Crozier in the middle twenties. The two curves both show a kink; again the previous authors have indicated the temperature at which they believed the kink occurs, and again this coincides nicely with the 15°C. anomaly in water.

It may be argued that perhaps it takes some "degree of faith" to draw lines of the type indicated and that the kinks may just be the result of a rather subjective curve fitting on the part of those who have analyzed the data. I would like to show another graph which I think will indicate that at least in some cases the kinks are not a matter of "sighting down the line to make it break at the right place." FIGURE 15 shows the protoplasmic

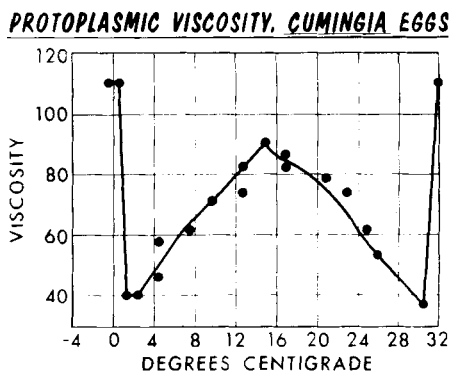


FIGURE 15. Viscosity of protoplasm for *Cumingia* eggs.

viscosity in *Cumingia* eggs as determined by Heilbrunn (1924). One sees that marked changes occur in the viscosity of the protoplasm in the vicinity of 2, 15 or 16°C., and again near 30 or 31°C. I do not pretend to understand in detail the significance of these marked changes, but they seem well above any criticism concerning "forced" curve drawing to give straight line segments deviating only slightly from each other. I am somewhat puzzled by the anomaly near 2°C.; however, the anomalies at 15 and 30°C. coincide very nicely with the anomalies in water and aqueous solutions. Not all examples are as dramatic as FIGURE 15; yet a very large amount of evidence indicates that the temperatures of the kinks in water are coincident with the marked changes in biological systems.

FIGURE 16 shows the relation between temperature and the frequency of division in *Chilomonas paramecium* (data by Smith, 1940). Again one

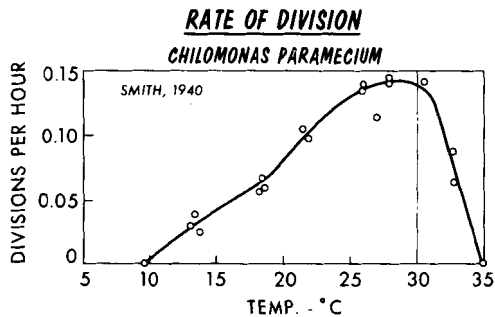


FIGURE 16. Frequency of cell division in the *Chilomonas paramecium* as a function of temperature.

notices a marked drop in the rate of cell division starting at 30 and vanishing at 35°C. Whether the anomaly indicated on the graph near 18°C. is a reflection of the 15°C. anomaly cannot be decided.

FIGURE 17 shows the time required for breakdown of the germinal vesicle of *Chaetopterus* eggs at various temperatures. These data are from a paper by Heilbrunn and Wilson (1955); the ordinate here is log of the time (in minutes) for breakdown of the vesicle, the abscissa is the temperature. Pronounced changes occur at both 12 and 30°C. It seems very reasonable to assume that the change at 30°C. reflects the kink at 30°C. in water; it is also likely that the anomaly at 12°C. reflects the 15°C. kink.

FIGURE 18 shows a study by Brown and Taylor (1938) of the excystment process for the ciliate *Colpoda duodenaria*. The abscissa is the reciprocal absolute temperature and the ordinate is the logarithm of the time for the excystment process. In this figure are shown the experimental points obtained by the author; the curve drawn, however, is what the present author

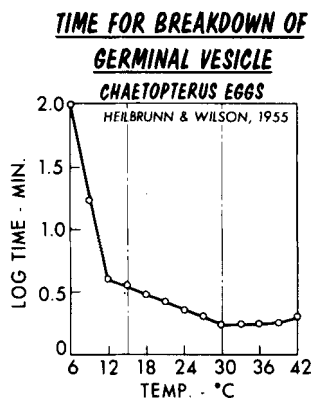


FIGURE 17. Time for breakdown of germinal vesicle of *Chaetopterus* eggs as a function of temperature.

considers a reasonably good fit to the data. Again one observes an anomaly near 15 and 30°C.

Complex Physiological Processes: Temperature Optima and Minima

As shown above, a number of "simple" kinetic phenomena in biologic systems show the occurrence of more or less abrupt changes at the temperatures of the kinks. We have now postulated (Drost-Hansen, 1956) that for a number of complicated biologic phenomena the existence of the structural transitions makes the biological system "prefer" to operate as far as possible from the temperatures of the kinks. Hence, we propose that in the process of evolution, biologic systems were selectively favored that operated in the middle of the interval between two consecutive transition

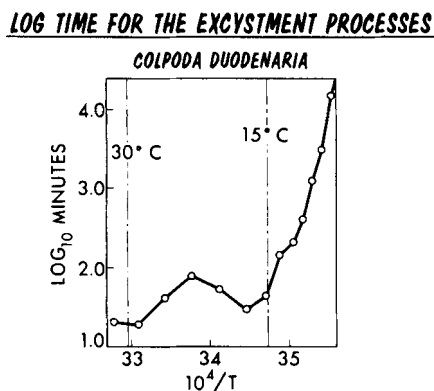


FIGURE 18. Rate of excystment for *Colpoda duodenaria*.

temperatures. Consider, for instance, the kinks at 30 and 45°C.: at either temperature the properties and behavior of a large number of individual processes may undergo rapid and “unpredictable” changes and hence it seems “safer” to operate the biological system near 37–38°C., in other words, as far removed as possible from either of the two kinks. Thus, we propose that in the course of evolution, temperatures near 37 and 38°C. have been favored by mammals as the optimum temperature for existence. FIGURE 19 shows a graph of body temperatures of approximately 160 mam-

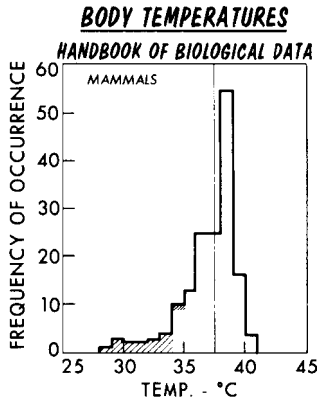


FIGURE 19. Distribution of body temperatures of mammals.

mals. From this graph it is seen that there is a strong predilection for body temperatures in the mammals between 37 and 39°C. The shaded area under the curve at the low temperature end indicates the body temperatures of such mammals as the anteater, the sloth, the echidna, the armadillo and a few other species of the type which, to a large extent, owe their existence only to freaks of nature, such as, isolation in unusual environments. Indeed, an animal like the duckbill platypus (body temperature around 30°C.) is a strange kind of a mammal in that it lays eggs, nests, feeds under the water as much as above the water, and in general does not seem to exhibit very predominantly mammalian behavior.

Further evidence of the importance of the kinks is the upper lethal temperatures. For all the mammals for which upper lethal temperatures have been established, it seems certain that 45°C. is an absolute upper lethal limit.

We have studied also the body temperatures of the birds. In this case, the agreement is not quite as impressive: the mean body temperature is around 41.5°C. However, it seems established that for the birds also, 45°C. constitutes an absolute, upper thermal limit. We may speculate as to the

cause for the displacement from 37 or 38°C. to near 41°C.; I should like to venture the guess that this is a compromise to the requirement of the highest possible energy production (such as we might predict from an Arrhenius type of activation mechanism) and the conformance to the structure and properties of water. In other words, the increased temperature may facilitate the production of energy necessary for flight while at the same time sacrificing "thermal latitude" such as resistance to infectious diseases (by minimizing the temperature range available for the bird in hyperthermia). It is interesting in this connection to note that most of the birds that do not fly, such as the ostrich, kiwi or penguin seem to have "normal" body temperatures namely between 38 and 39°C.

It is well known that in both man and many other mammals, 30°C. is a temperature of considerable physiological importance. For example, in man there is loss of consciousness below 30°C., together with a loss of the ability to regulate body temperature. Moreover, below 30°C., marked changes occur in the oxygen consumption of various tissues (this being one of the reasons why cardiovascular surgery is sometimes performed under hypothermia, often at temperatures around 27 or 28°C.). FIGURE 20

OXYGEN UPTAKE IN RABBITS

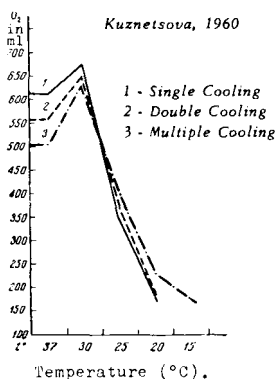


FIGURE 20. Rate of oxygen consumption of rabbits; (By permission — Pergamon Press).

shows an example of change in oxygen consumption. The ordinate is the oxygen uptake of rabbits, the abscissa is the temperature (Kuznetsova, 1960); the rate of O_2 -uptake drops abruptly below 30°C.

By analogy with the systems enumerated above, we suggest that optima between 45 and 60°C. should exist for other types of living organisms. Indeed, many thermophilic bacteria are known to possess optima around

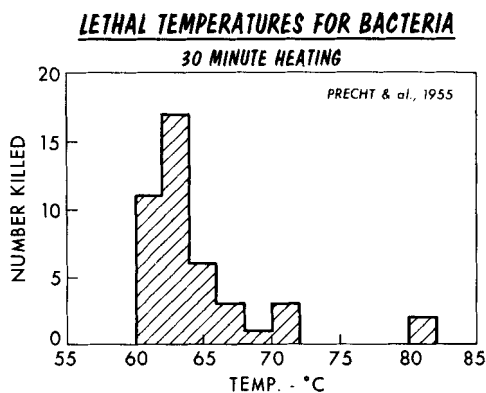


FIGURE 21. Lethal temperatures for a number of bacteria under various conditions (heating time: 30 minutes).

53–55°C., pasteurization temperatures usually tend to be approximately 60 to 62°C. FIGURE 21 shows the lethal effects of heating above 60°C. for a compilation of 20 different bacteria under a variety of different conditions, mostly different media. The data shown are all those cases for which heating times of 30 minutes have been used; this histogram has been drawn from data compiled by Precht, Christophersen and Hensel (1955).

The same type of argument as presented above can be applied to the interval between 15 and 30°C. Here we find optimum activity near 22 to 25°C. for a large number of vastly different types of animals: many insects (though not all), many fishes, and many soil bacteria seem to have optima in the vicinity of 23 to 25°C. Also, 30°C. is known to be an important temperature physiologically for both fishes and insects. We shall illustrate this shortly with a number of specific examples. Furthermore, it is known that 15°C. often is a controlling factor in ecology: for example, in the distribution of fishes in the South Pacific (Jones, 1947) where the density of fishes of commercial interest drops precipitously at temperatures below 15°C. Another example of ecologic interest is shown in FIGURE 22 where the ordinate is the rate of egg deposition of the gastropod *Urosalpinx cinerea* and the abscissa is the temperature. This illustration, which is redrawn from the data by Moore (1958) shows that 15°C. is indeed a critical temperature below which no deposition of eggs take place.

Some Kinetic Aspects and Examples

As outlined above, there exist in water more or less abrupt structural changes that give rise to changes in the properties of pure water and in aqueous solutions. I believe that these changes significantly influence the behavior of biologic systems. Obviously, it is not claimed that these changes

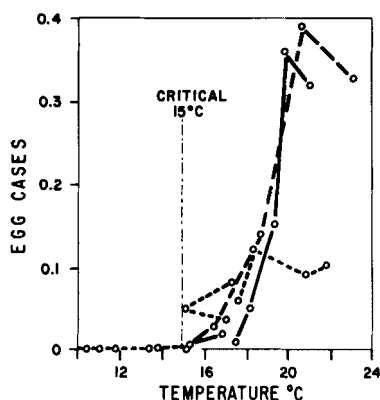
EGG DEPOSITION OF GASTROPOD (*UROSALPINX CINEREA*)

FIGURE 22. Rate of egg deposition of gastropod.

determine all the thermal properties of biological systems but the structural changes in water can certainly account for at least some of the anomalous and often abrupt changes encountered in biologic systems.* Thus, these changes may suggest reasons for tendencies in optimum temperature selections in biological systems. While I do not in general take issue with the formulations of the kinetics of biologic systems, as developed, for instance, by Johnson, Eyring and Polissar (1954), I believe that the structural changes in water must also be considered as important constraints on the biologic systems and that these constraints superimpose on other mechanisms so as to greatly affect the systems' over-all temperature-dependence. The notion of the kinks can assist us significantly in understanding the behavior of many biologic systems where, for instance,

*One might inquire as to how often the anomalies show up at the "right temperatures" in the biological systems; — "right temperatures" meaning, say, within 2 to 3°C. of the temperature of the kinks in water and aqueous solutions. Based on all available data, the author is of the opinion that the "agreement" at least holds for something like 75 out of 100 cases (this does not include the problem of body temperatures of mammals and birds covered in the preceding section). The general validity of this estimate, however, depends on the reliability of the sampling of examples and it may be argued that perhaps the author and his associates have chosen only those examples which "fit" the notions expressed above at the expense of examples which do not support the theory (either by showing no inflection points, maxima or minima, or by having the kinks at the "wrong" temperatures). Initially, we were indeed looking for specific examples which could be used to support our original hypothesis — this is the way one goes about constructing empirical theories. Over the years, however, we have tried to exercise appropriate objectivity although the possibility remains that we may be "subconsciously" prejudiced. At least, we are aware of the problem.

Johnson, Eyring and Polissar's theory alone is not likely to provide all the answers. One example is the observation made by Andrewartha and Birch (1954), based on an analysis by Moore, that for 41 aquatic animals (*Crustacea*, *Echinodermata*, *Mollusca*, *Tunicata* and *Pisces*), 91 per cent of them were restricted to a range of 16°C. or less (for 42 per cent of them, the range was 14°C. or less). It is interesting that this activity interval of 16° coincides very closely with the interval between consecutive kinks, optima for the activity of fishes often being approximately midway between 15 and 30°C. As an example, FIGURE 23 shows the distribution of

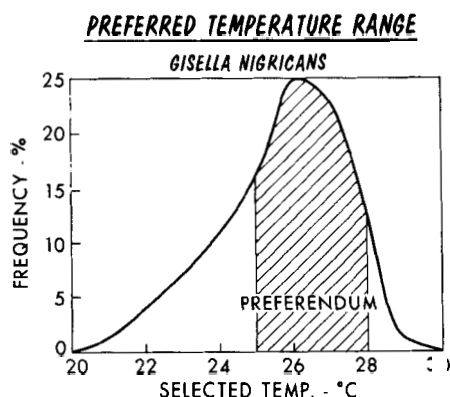


FIGURE 23. Preferred temperature ranges for the fish, *Gisella nigricans*.

preferred temperatures for the fish *Gisella nigricans* as a function of temperature (See Andrewartha and Birch, 1954). About 75 per cent of the fish congregate between 25 and 28°C., with no fishes preferring temperatures above 30 or below 20°C.

FIGURE 24 shows the temperature ranges for normal development of eggs of the meadow frog, *Rana pipiens*, from different localities in North America (see Andrewartha & Birch, 1954). Again one observes that 30°C. is, with a few exceptions, as high as the normal development is encountered. Note also that 15°C. does not seem to be nearly as important a temperature for the frog as 30°C.

FIGURE 25 shows the logistics curve for development per hour in per cent of the egg stage of *Calandra oryzae* (Andrewartha & Birch, 1954) again the abscissa is the temperature. The logistics curve is very accurately followed in the range from 15.5 to 30°C., but for temperatures of 30 to 34°C., very marked deviations occur.

FIGURE 26 shows the "calculated" and observed development curves for *Drosophila melanogaster* in the pupa state (see Davidson, 1944). Again,

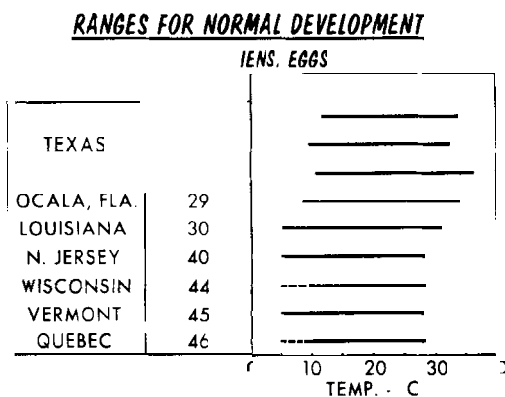


FIGURE 24. Ranges of temperature for normal development of eggs of the meadow frog, *Rana pipiens*.

the behavior follows closely the logistics curve between 15 and 30°C., but deviations occur above 30°C. The logistics curve for the egg state is almost identical in shape. This points to an observation that we have often made. The properties of water are alleged to be very anomalous and indeed this is so. However, in studying the behavior of solutions we often find that "normal" behavior is encountered as long as attention is restricted to the properties between consecutive kinks. For instance, plotting logarithm of solubility or logarithm of reaction rates versus the reciprocal, absolute temperature often gives to a very good approximation "perfectly straight lines" between say 15 and 30°C. or between 30 and 45°C. Within these intervals the behavior is indeed "normal." Hence, we should perhaps not

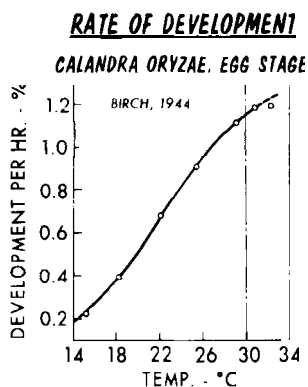


FIGURE 25. Logistics curve for development of the egg of *Calandra oryzae*.

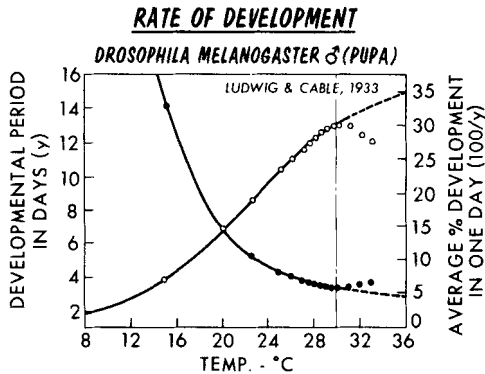


FIGURE 26. Logistics curve for development of the pupa of *Drosophila melanogaster*.

be too surprised to find, as we did, that the development of the egg stage of the insect follows very nicely a moderately simple logistics curve. On the other hand, we should expect the marked anomalies that, in fact, occur near the kink at 30°C.

Multiple Temperature Optima

If we are correct in assessing the importance of the structural changes in water for the behavior of biologic systems, it may be possible to delineate ranges of environmental temperatures that are conducive to life. Life generally cannot be maintained for any organism over a wide range of temperatures. Andrewartha and Birch, state, for instance: "...no individual species is known which can thrive over such a wide range as from 0 to 50°C." This statement is probably generally quite true, however, in our own studies we have come upon bacteria that have multiple ranges for optimal growth.

We have studied a sulfate-reducing bacteria, probably a *clostridium*, that was able to grow over a temperature range of from 7 to 45°C., with three separate ranges of optimum activity (Oppenheimer & Drost-Hansen, 1960). FIGURE 27 shows the initial results obtained in this study; growth optima are seen to occur near 12, 26 and 39°C. Because the initial study was performed using only visual estimation of the turbidity as a means of estimating the growth, we have since repeated this experiment and checked the amount of growth with the spectrophotometer (Schmidt & Drost-Hansen, 1960); FIGURE 28 shows the results. Again, optimum activity occurs near 24 and 40°C., with growth minima near 15, 30 and above 45°C. In this particular experiment the activity did not drop to zero at the 30°C. kink, but the optical density does go through a minimum near this temperature. Unfortunately, the experiment was not conducted at sufficiently

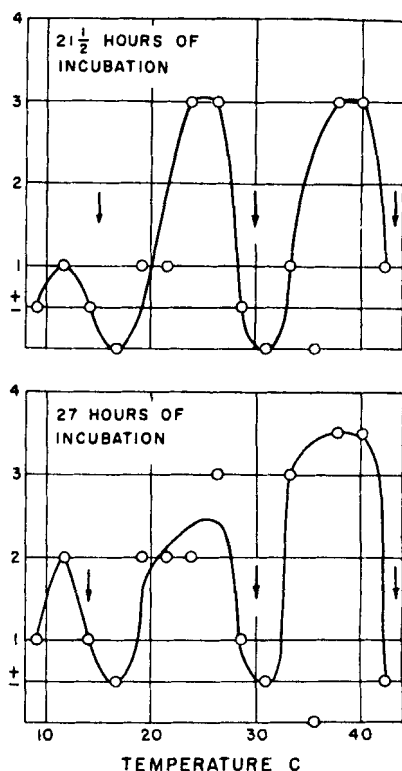


FIGURE 27. Growth of a sulphate-reducing bacterium, probably a *clostridium*.

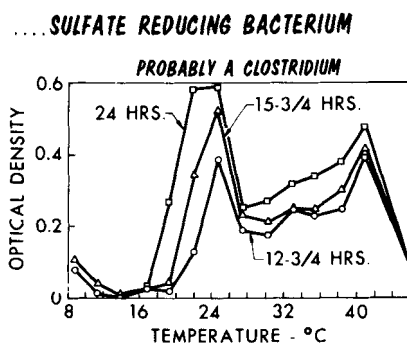


FIGURE 28. Growth of a sulphate-reducing bacterium, probably a *clostridium*, as determined by optical density measurements with spectrophotometer.

GROWTH OF NEUROSPORA - MUTANT

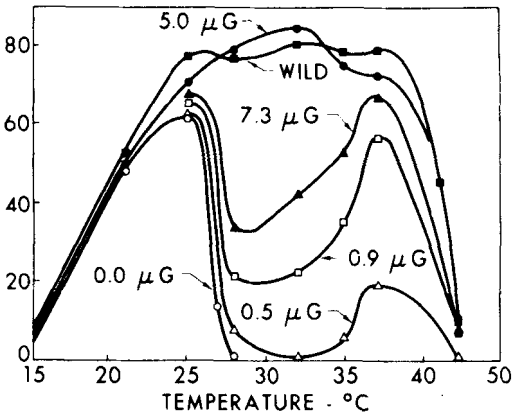


FIGURE 29. Growth of mold; a mutant of *Neurospora crassa*.

low temperatures to determine if a third optimum existed below the 15°C. minimum.

A similar type of growth curve was reported earlier by Mitchell and Houlahan (1946), who studied the growth of a *Neurospora crassa* mutant that was made to require lactoflavin for growth. The amount of growth (determined as dried weight of the cells) is shown in FIGURE 29 as a function of temperature in the presence of the amounts of lactoflavin indicated on the curves. We are here dealing with an organism that for small concentrations of lactoflavin has distinct growth minima near 15, 30 and 45°C., with optima near 23 and 37°C. These are exactly the type of results we

.....UNBUFFERED MEDIUM, NO DEXTROSE

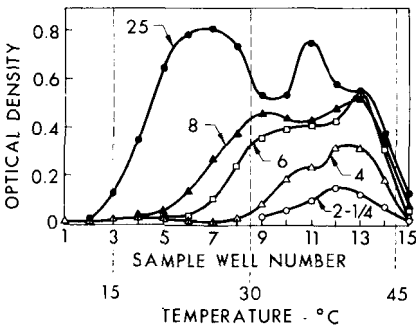


FIGURE 30. Growth of *E. coli* as a function of temperature for five different times of growth.

would predict from our considerations of the influence of the kinks on the biological systems.

Further corroboration of these predictions is given by a more recent study of the growth of *E. coli* (Schmidt & Drost-Hansen, 1961). FIGURE 30 shows the amount of growth, measured as optical density, at five different times of growth as a function of temperature. In the older cultures there are again two optima for growth, namely near 26 and 35°C. FIGURE 31

.....UNBUFFERED MEDIUM, NO DEXTROSE

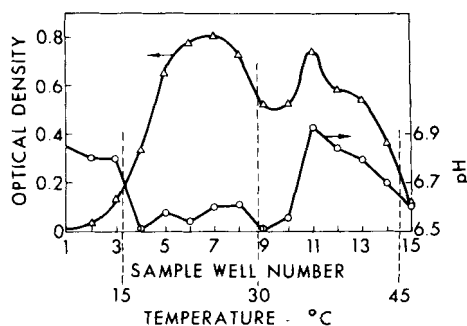


FIGURE 31. *pH* of system shown in FIGURE 30 at the end of 25 hours of growth.

shows the measured *pH* of the system at the end of 25 hours of growth: between 15 and 30°C., the *pH* dropped to 6.6, while below 15°C. and above 32°C., *pH* was increased over the initial value. We have observed this type of behavior on several occasions. In FIGURES 28 and 30, the two peaks and the minimum between them suggest the superposition of two growth peaks that overlap to some extent. We offer the accompanying *pH* changes as evidence for a hypothesis formulated earlier (Oppenheimer & Drost-Hansen, 1960): that some organisms are able to grow optimally in different temperature intervals by utilizing different metabolic pathways. We have also obtained evidence from a study of *Serratia marcescens* (Schmidt & Drost-Hansen, 1960) that the well known difference in red pigment formation changes near 30°C. This, perhaps, is another manifestation of different metabolic pathways utilized by the organism for growth above and below 30°C.

Discussion

Evidence has been presented for the existence of thermal anomalies in the properties of water. The anomalies probably reflect higher order phase-transitions or some other order-disorder phenomenon involving cooperative action on the part of the water molecules. On this basis, one may then

suggest that the structure of water must be characterized by cooperating units whether they be cages or clusters. Very likely, the sizes of the cooperating units are between 10 to 200 molecules; the lower limit is determined very roughly by the number of particles necessary to obtain cooperativeness, and the upper limit seems reasonable, since, were the clusters much larger, they would have manifested themselves quite readily in optical scattering, X-ray and neutron diffraction measurements and presumably in several other types of measurements. Recently some direct evidence has been reported of "clusters" in water in the size range mentioned (Egelstaff, 1962). It has also been argued that the kinks remain unchanged in the presence of most any solute and that this must imply the persistence of structural features characteristic of pure water in the presence of solutes. Hence, it was a simple matter to extend the notion of the existence of the kinks to biologic systems that are indeed aqueous solutions with, albeit, large surface-to-volume ratios.

It remains to be explained how the kinks may play such a prominent role in biologic systems when they are oftentimes quite difficult to determine, for instance, from data on the properties of pure water. The explanation may possibly be related to the fact that the biologic phenomena of interest to us take place over a considerably narrower temperature range than that over which we are able to study ordinary physicochemical systems. Hence, with equal diligence on the part of the physical chemist and the biologist, the biologist may "squeeze in", say, 10 measurements over a range of 20 to 30°C. — the total range of interest in nondestructive studies of an organism or a protein solution — while the physical chemist may well be interested in the properties extending from far below 0°C. to considerably above the boiling point. Hence, we simply have more data available at more closely spaced temperature intervals for biologic systems than are available for most physico-chemical parameters.

In addition to the "numerical" advantage of the biologic examples, we may perhaps speculate that such phenomena as hydrophobic bonding of water around a protein molecule or ordering of water molecules adjacent to a biologic membrane may constitute far more sensitive probes of the aqueous environment than the behavior of, say, an alkali ion or any other "single", "small" solute species in water. The cooperative action between many water molecules in the water clusters of the solvent water may well be expected to influence drastically the rather larger amount of water associated with the proteins or membrane material. In other words, the structural transitions in water may exert a direct and profound influence on the immediate environment of the macromolecules of the biologic systems; the effects of the transitions are not merely "solvent effects" manifested by minute changes in the solvent viscosity, dielectric properties or activity. Except for vaguely defining some cooperating units such as

clusters or cages we are able to appreciate structural peculiarities of water in biologic systems without a more specific model.

In a recent paper, Davey and Miller (1965) at North Carolina State University have provided important experimental evidence for the reality of growth minima for microorganisms in the vicinities of the temperatures of the kinks in pure water. We quote their abstract: "Water in the liquid phase has been shown, by others, to undergo subtle changes in physical structure near 15, 30, 45 and 60°C. Four bacteria were used to cover the range in temperature from 5 to 70°C. in order to determine whether these temperature-dependent anomalies in the structure of water may have biological implications. In all cases growth was suppressed at the predicted temperatures. This suggests a strong interaction between the structure of water and biological activity." Davey and Miller also noted that anomalies at temperatures other than the four predicted ones were not observed (between 5 and 70°C.) and that the observed temperature-related anomalies in the growth of the four bacteria seemed to be greater in magnitude than in most, if not all, of the published physical data, indicating the highly sensitive nature of biologic systems to subtle alterations in their micro-environment.

Conclusions

There appear to exist in water and aqueous solutions thermal anomalies reflecting cooperative order-disorder phenomena in the water structure. It is suggested that these structural anomalies significantly influence the behavior of biologic systems, specifically that they may account for the existence of more or less abrupt changes with temperature in many biologic phenomena and that they delineate optimum and minimum temperatures for biologic activity.

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