Physiol. Chem. Phys. & Med. NMR (2008) *40*: 115–118

A Preliminary Report on the Survival of Fully-hydrated Living (Cancer) Cells to Liquid Helium Exposure

Gilbert N. Ling and Margaret M. Ochsenfeld

Damadian Foundation for Basic and Cancer Research, Fonar Corporation,
110 Marcus Drive, Melville, NY 11747
Email: gilbertling@dobar.org

Abstract: Fully-hydrated Ehrlich carcinoma ascites cells under the protective action of DMSO fully survived exposure to near-absolute zero temperature provided by liquid helium.

KEY WORDS: association-induction hypothesis, AI hypothesis, Bradley isotherm, cell water, DMSO, fully-hydrated cells, idealized NP system, liquid helium temperature, NaCl exclusion, non-freezing water, polarized-oriented multilayer theory, PM theory, POM theory, 0°K.

THE YEAR 1965 saw the addition to the theory of the living cell called the association-induction hypothesis (Ling 1962; 1997; 2001), the *polarized-oriented multilayer* (POM, or PM) theory of cell water. The POM theory was introduced to explain the low level of sodium ion (Na^+) as chloride (and other solutes like sucrose) in cell water (Ling 1965.) To achieve that end, the theory contends that all or virtually all cell water in living cells is polarized and oriented by the cell proteins. For many years afterwards, Bradley's multilayer adsorption isotherm has offered important support for the POM theory (Bradley 1936.) Notwithstanding, this and other then-existing theories had limitations. Thus, they could not provide key quantitative insights needed, including the depth of multilayers that can be so polarized (and oriented) by the polar surfaces (Ling 2003.)

By taking a short cut, Ling derived a new theoretical foundation for his POM theory of cell water and inanimate systems demonstrating long range dynamic structure. The theory began by defining and calling a checkerboard of positively- and negatively charged sites of a rigorously defined geometry and dimension as an *idealized NP system.* The theory then shows that under idealized conditions — including 0°K temperature, freedom from any kind of disturbance etc. — the propagated polarization-orientation of bulk-phase water molecules produced by such an idealized NP system could proceed *ad infinitum.*

In addition, the theory predicts that water so polarized and oriented would not freeze at any attainable low temperature. This prediction was confirmed retroactively some fifty years before when Canadian chemists, Giguère and Harvey discovered unintentionally that thin films of water held between polished silver chloride lenses — which possess crystal structure nearly ideal as defined in the new theory — would not freeze at liquid nitrogen temperature (–196°C or 77° K.) (Giguère and Harvey 1956)

Following the introduction of the POM theory in 1965, unusual properties of water in the living cell and model systems beyond the low solvency for NaCl were examined one by one in the light of the new theory. (For a full list of these attributes, see Ling 1992, Table 5.5 on pp. 208–209.) One of these additional attributes is the demonstrated ability of living cells to survive freezing and thawing in liquid nitrogen when a *cryoprotective agent* like glycerol or dimethyl sulfoxide (DMSO) was added to the freezing medium (for review, see Smith 1961.) And in time, the predictions of the POM theory was confirmed not only for these two physiological manifestations — Na(Cl) exclusion and survival in freezing temperature — but for six other manifestations listed in the table quoted above as well.

Ling and Zhang studied the freezing and thawing behaviors of two categories of model systems referred to as the extrovert and introvert respectively (Ling and Zhang 1983.) Extrovert models include proteins that for structural reason, like gelatin, or in response to denaturants, like urea, exist in the *fully-extended* state and various linear oxygen carrying polymers including polyethylene oxide, polyvinylpyrrolidone. Introvert models include mostly what are often (erroneously) called "native" proteins. In most of our studies with a Perkin Elmer differential scanning calorimeter, the low temperature was provided by a mixture of dry ice and ethanol. In a smaller number of runs, we used liquid nitrogen. The lowest temperature reached in our runs was around 123° K.

From these model studies, Ling and Zhang confirmed most if not all of what was theoretically predicted. Thus, in solutions up to as high as 50% concentration, the six introrvert "native" proteins investigated demonstrated no detectable influence on either the freezing temperature of the bulk phase water or the width of its freezing peak — in a plot of heat absorption or release against increasing (or decreasing) temperature. In contrast, all the extrovert models uniformly demonstrate strong influence on the freezing (and thawing) pattern of the bulk-phase water. Thus with increasing polymer concentration, the freezing temperatures became steadily lower and the freezing peak became progressively wider — so that at the highest polymer concentration, the freezing peak disappeared altogether. The vanishing freezing peak shows that a state of non-freezing had been brought about by the extrovert models.

That extrovert models can lower the freezing temperature of the bulk phase water and make it unfreezable is important. It demonstrates that closer juxtaposition of water-polarizing and orienting sites enhances the stability of the dynamic polarized-oriented water. As such, it offers an explanation why glycerol, dimethyl sulfoxide (DMSO) and other cryoprotectants help to preserve living cells from fatal injury from freezing and thawing: Because these cryoprotectants as a rule form strong H-bonds with water molecules and by so doing further enhance the water-to-water interaction energy of the bulk phase water in the resting cell by the fully extended protein chains and make the cell water unfreezable. (See also Ling, Niu and Ochsenfeld 1993, pp. 193–195; Ling 2006)

That increasing the concentration of the extrovert models could cause non-freezing also offers an explanation why only after severely lowering of the water content (to 8%) could

the larvae of the West African beetle, *Polypedilum vanderplanki* survive exposure to liquid helium (Hinton 1960.)

The purpose of this preliminary study is to find out if living cells in their normal fully hydrated state could also survive exposure to liquid helium when the cells are under the protection of the cyoprotectant, DMSO (Lovelock and Bishop 1959.) If this proves successful, it would go one step further confirming the theory of non-freezability at *any* attainable low temperature when the bulk phase water is polarized and oriented like that in the idealized NP system.

Materials and Methods

Ehrlich carcinoma ascites cells were carried in ICR mice and routinely harvested 14 days after abdominal injection. The ascites fluid was mixed with Sigma RPMI 1640 medium before low speed (900 rpm) centrifugation and resuspension of the cells in similar medium and centrifugation a second time. 0.5 to 0.7 gram of the cell pellet was then suspended in 5 ml of a freezing medium (76% RPMI medium, 16% newborn calf serum and 8% DMSO.) The suspension was then further diluted with similar freezing medium by mixing 0.85 ml of the suspension in 20 ml of the freezing medium and 2 ml aliquots of the suspension placed in 2 ml sterile cryo-tubes. After 2 hours of gentle shaking in a refrigerator kept at about 9° C, the cryotubes containing the ascites cells were suspended over-night inside a standard 10 liter liquid nitrogen tank at about 13 inches below the top of the tank and about 6–7 inches from the surface of the liquid nitrogen beneath. The cells in the cyrotubes were then lowered into the liquid nitrogen.

To expose the ascites cells to near absolute zero temperature, we allowed liquid helium to flow over the cryotubes — containing the liquid-nitrogen frozen ascites cells — in a Dewar jar for a total duration of 5–10 minutes.

Results and Discussion

The viability of the ascites cells were examined after staining with erythrosin B. Sample cells taken before freezing in liquid nitoroten was 97.7% ; it fell to 81% after freezing in liquid nitrogen. Following the exposure to liquid helium, the viability stayed essentially unchanged at 86% survival.

Before exposing to the liquid helium, the ascites cells had been in liquid nitrogen four days at the temperature of –196° C or 77.2° K. Liquid heliium could provide a temperature of 1.9° K as it was maintained at the Large Hadron Colllider at CERN in Switzerland. The usually given value is 3 to 4° K. The temperature our samples sank to was unknown but it is certainly much lower than 77° K. The fact that the viability remained entirely undiminished by further lowering of temperature gives us the assurance that the theoretical prediction that living cells in the presence of cryoprotectant DMSO could indeed survive temperature close to the temperature of liquid helium and hence absolute zero.

We thank Dr. Raymond Damadian and the Fonar Corporation for their continued support.

References

Bradley, R.S. (1936) Polymolecular Adsorbed Films. Part II. The General Theory of the Condensation of vapors on finely divided solids. *J. Chem. Soc.* 1936: 1799–1804.

Giguère, P.A. and Harvey, K.B. (1956) On the infrared absorption of water and heavy water in condensed states. *Canad. J. Chem*. 34: 798–808.

Henniker, J.C. (1949) The depth of the surface zone of a liquid. *Reviews of Modern Physics* 21: 322–341.

Hinton, H.E. (1960) A fly larva that tolerates dehydration and temperture of –270 C to +102 C. *Nature* 188: 333–337.

Ling, G.N. (1962) *A Physical Theory of the Living State: the Association-Induction Hypothesis*, Blaisdell Publ. Co., Waltham, MA.

Ling, G.N. (1965) The physical state of water in living cells and in model systems. *Ann. N.Y. Acad. Sci.* 125: 401–417.

Ling, G.N. (1992) *A Revolution in the Physiology of the Living Cell,* R. Krieger Publ.Co., Malabar. FL

Ling, G.M\N. (1997) http://www.gilbertling.org

Ling, G.N. (2001) *Life at the Cell and Below-Cell Level: the Hidden History of a Fundamental Revolution in Biology,* Pacific Press, NY.

Ling, G.N. (2003) A new theoretical foundation for the polarized-oriented multilayer theory of cell water and for inanimate systems demonstrating long-range dynamic structuring of water molecules. *Physiol. Chem. Phys. & Med. NMR* 35: 93–130. Also available at: http://www.physiologicalchemistryandphysics.com/pdf/PCP35-91_ling.pdf Or go to www.gilbertling.org choose volume and article from drop-down list and click.

Ling, G.N. (2006) Chapter 1. A convergence of experimental and theoretical breakthrough affirms the PM theory of dynamically structured cell water at the theory's 40th birthday in: *Water and the Cell (Pollack, G.H., Cameron, I.L. and Wheatley, D.N., eds.) Springer Verlag, Berlin, New York.* Can also be reached by going to www.gilbertling.org and click the title on the front page of the website.

Ling, G.N. and Zhang, Z. (1983) Studies on the physical state of water in living cells and model systems. IV. freezing and thawing point depression of water by gelatin, oxygen-containing polymers and urea-denatured proteins. *Physiol. Chem. Phys. & Med. NMR* 15: 391–406. Also available at: http://www.physiologicalchemistryandphysics.com/pdf/PCP15-3911_ling_zhang.pdf Or go to www.gilbertling.org choose volume and article from drop-down list and click

Ling, G.N., Niu, Z. and Ochsenfeld, M. (1993) Predictions of polarized multilayer theory of solute distribution confirmed from a study of the equilibrium distribution in frog muscle of twenty-one nonelectrolytes including five cryoprotectants. *Physiol. Chem. Phys. & Med. NMR* 25: 177–208. Also available at: http://www.physiologicalchemistryandphysics.com/pdf/PCP25-177_ling_niu_cohsenfeld.pdf Or go to www.gilbertling.org choose volume and article from drop-down list and click.

Lovelock, J.E. and Bishop, M.W.H. (1959) Prevention of freezing damage of living cells by dimethyl sulfoxide. *Nature* 183: 1394–1395.

Smith, A. U. (1961) *Biological Effects of Freezing and Supercooling*, Williams and Wilkins, Baltimore.

Received August 5, 2009;
accepted September 10, 2009