

This Week's Citation Classic[®]

CC/NUMBER 44
NOVEMBER 1, 1993

Yancey P H, Clark M E, Hand S C, Bowlus R D & Somero G N. Living with water stress: evolution of osmolyte systems. *Science* 217:1214-22, 1982.

[Whitman Coll., Walla Walla, WA; San Diego State Univ., CA; Univ. Southwestern Louisiana, Lafayette, LA; Harvard School, Los Angeles, CA; and Scripps Institution of Oceanography, La Jolla, CA]

Cells exposed to long-term water stress raise osmotic pressure with a few types of organic solutes. Unlike salts, these osmolytes are compatible with or stabilize proteins and membranes. Originally found in organisms in saline waters, they have since been found in systems as diverse as soil bacteria, desert plants, and the mammalian brain and kidney. Research has shown them to be involved in cryopreservation, diabetes, and the Gaia hypothesis. [The SC[®] indicates that this paper has been cited in more than 400 publications.]

Micromolecules That Help Macromolecules in Dehydration

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When I joined George N. Somero's lab at the Scripps Institution of Oceanography in 1974, he was talking with Mary E. Clark of San Diego State University about evolution in a way not taught in my biology education. Their framework was the selection of the intracellular milieu and the crucial *micromolecules*—inorganic ions, small organic solutes, and H₂O—that define it. Biologists, they felt, were learning much about the function and evolution of macromolecules, but little on the nature of the environment in which they operated. A major question: Why are inorganic ion concentrations in cells limited to a fairly narrow range in most organisms? Most were known to accumulate costly organic solutes rather than salts. George and Mary noted from the literature that only a few types of solutes were used as osmolytes: some carbohydrates (e.g., polyols in marine algae), neutral free amino acids (e.g., most marine invertebrate phyla), and methylated amino compounds (e.g., many marine groups). Why?

Mary (and, independently, Wyn Jones in Wales) reasoned that amino acids are used because they are similar to certain inorganic salts (e.g., NH₄⁺, CO₂²⁻), long known to stabilize protein structure.¹ Unlike common cell ions (K⁺, Na⁺, Cl⁻), stabilizers might be raised to high levels without disturbing protein function. A.D.

Brown and colleagues in Australia had that idea for polyols,² after finding that high Na or KCl levels inhibit proteins of salt-tolerant and intolerant algae, while glycerol (the main osmolyte of the former) does not. They proposed that these were general properties of protein-solute-water interactions, rather than specific protein adaptations for function with osmolytes, coining the term "compatibility" for nonperturbation. Mary, in her lab, and Dave Bowlus, in George's lab, were testing amino acid osmolytes and were finding similar compatibility properties.

At that time, I noted that one osmolyte had not been explained: urea. This waste/osmolyte occurs up to 500m M in cartilaginous fishes (sharks, etc.), and as a well-known protein denaturant seemed clearly noncompatible. In dissertation work, I found that most enzymes from these fish were not adapted to work well in urea. However, sharks also have high levels of methylamine osmolytes (e.g., trimethylamine oxide). Inspired by Mary, I noted that these resembled the best protein-stabilizing cation, (CH₃)₄N⁺. My studies (later extended by Steve C. Hand) showed that urea and methylamines have opposite and offsetting effects on protein structure and function. George and I termed this net compatibility "counteraction."

In 1980, while I was doing postdoctoral work in Scotland and Norway, George urged that we synthesize the numerous independent studies on osmolytes in various water stress conditions. Our resulting paper in *Science* has shown great longevity because it was the first complete review to cover universal properties, types, and distribution of osmolytes. Research (including my own) has expanded in the last decade;¹ e.g., in 1985 at the NIH, osmolytes like those in marine organisms were found in the mammalian kidney (with its high urea and salt levels^{1,3}), a discovery directly inspired by our paper,⁴ with possible medical importance.^{3,5} Other practical potentials include *in vitro* preservation¹ and engineering crops for saline water.⁸ And, a major osmolyte of marine phytoplankton has been called a key regulator of Earth's climate via dimethylsulfide production and the cloud formation it may trigger (in the Gaia hypothesis⁷)—an osmolyte system that may be crucial for our planet's future!

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Received October 23, 1992