Perspectives on: The response to osmotic challenges

Olaf S. Andersen

Department of Physiology and Biophysics, Weill Cornell Medical College, New York, NY 10065

The purpose of the Perspectives in General Physiology is to provide a forum where scientific uncertainties or controversies, or important problems, are discussed in an authoritative, yet open manner. The Perspectives are solicited by the editors—often based on recommendations by members of the editorial advisory board. To frame the issue, two or more experts are invited to present brief points of view on the problem; these are published consecutively in the Journal. One or more experts and the organizer review the contributions, but the comments and opinions expressed in the Perspectives are those of the authors and not necessarily those of the editors or the editorial advisory board. The Perspectives are accompanied by a few editorial paragraphs that introduce the problem and invite the submission of comments, in the form of letters to the editor, which are usually published four months after publication of the Perspectives. After the letters to the editor have been published, further responses are limited to full manuscripts.

In this issue, Frederick Sachs and Mettupalayam V. Sivaselvan (University of Buffalo), Janet M. Wood (University of Guelph), and Elizabeth S. Haswell and Paul E. Verslues (Washington University, St. Louis, and Academia Taiwan, Taipei) provide different perspectives on how cells respond to osmotic challenges.

Life occurs in water, which constitutes about two thirds of the volume of most organisms. Living cells, whether single-celled organisms or the cells in multi-cellular organisms, exist in an aqueous environment, and there is continuous movement of solutes (nutrients, electrolytes, and metabolic waste) and water across the membranes that separate cells from their environment. All cell membranes are permeable to water, and water occupies volume, so cells need to regulate their volume in response to changes in their environment—including osmotic challenges. The challenges, however, are different for plants and many bacteria, which are enveloped by a (rather) rigid cell wall.

The fundamental principle underlying this regulation is that, for water to be in equilibrium, the chemical potential of water (μ_w) must be the same throughout the space to which it has access, e.g., Finkelstein (1987). In the case of cells, μ_w must be the same in the extracellular (e) and intracellular (i) compartments:

$$\mu_W(i) = \mu_W(e), \tag{1}$$

For dilute solutions, μ_w can be expressed as:

$$\mu_W = \mu_W^0 + RT \cdot \ln\{x_W\} + P \cdot \bar{V}_W, \tag{2}$$

where μ_W^0 denotes the standard chemical potential of water, R is the gas constant, T is the temperature in kelvin, x_w is the mole fraction of water, P is the hydrostatic pressure of the solution in question, and $\bar{V}_W$ is the partial molar volume of water. (In more concentrated, nonideal solutions, including the cytoplasm where molecular crowding becomes important [Luby-Phelps, 1999; Dix and Verkman, 2008; Mika and Poolman, 2011], it will be necessary to introduce an activity coefficient for water [γ_w] in Eq. 2.) Combining Eqs. 1 and 2, water will be in equilibrium when

$$\mu_W^0 + RT \cdot \ln\{x_W(i)\} + P(i) \cdot \bar{V}_W = \mu_W^0 + RT \cdot \ln\{x_W(e)\} + P(e) \cdot \bar{V}_W, \tag{3}$$

or

$$P(i) - P(e) = \Pi = -\frac{RT}{\bar{V}_W} \cdot \left(\ln\{x_W(i)\} - \ln\{x_W(e)\}\right), \tag{4}$$

where Π is the osmotic pressure difference between the intra- and extracellular compartments—the hydrostatic pressure difference that must exist between the two compartments to have no net water movement across the membrane. Using the relations

$$x_W = \frac{n_W}{n_W + n_S} = 1 - \frac{n_S}{n_W + n_S} = 1 - x_S \approx 1 - \frac{n_S}{n_W} \text{ and } \frac{n_S}{\bar{V}_W \cdot n_W} \approx C_S,$$

where n_s and n_w denote the number of moles of solute (sum of the number of moles of all solute particles) and water, and C_s is the solute concentration, Eq. 4 becomes

$$\begin{aligned} \Pi &= -\frac{RT}{\bar{V}_W} \cdot \left(\ln\{1 - x_S(i)\} - \ln\{1 - x_S(e)\}\right) \\ &\approx \frac{RT}{\bar{V}_W} \cdot \left(x_S(i) - x_S(e)\right) \approx RT \cdot \left(C_S(i) - C_S(e)\right), \end{aligned} \tag{5}$$

Correspondence to Olaf S. Andersen: sparre@med.cornell.edu

Abbreviation used in this paper: RBC, red blood cell.

The Rockefeller University Press
J. Gen. Physiol. Vol. 145 No. 5 371–372
www.jgp.org/cgi/doi/10.1085/jgp.201511397

which is the Van 't Hoff expression. That is, the osmotic pressure difference does not result from the pressure exerted by solute particles on the walls of the compartment enclosing the solute particles (as is sometimes assumed based on the similarities between Eq. 5 and the ideal gas law); indeed, the hydrostatic pressure in the solution with the lower solute concentration may be negative, as first deduced by Lars Vegard (1908); see also Mauro (1965, 1979).

If a cell is in osmotic equilibrium with its extracellular solution ($C_S(i) = C_S(e)$), and the extracellular osmolality suddenly is decreased (or increased), water will flow into (or out of) the cell and thereby change its volume until a new equilibrium state has been reached—with $\mu_W(i) = \mu_W(e)$, where the changes in $\mu_W(i)$ could result from the inevitable changes in $C_S(i)$ as well as from changes in the pressure difference across the cell membrane.

Human red blood cells (RBCs), which have only a submembraneous (or cortical) cytoskeleton, have long been a favorite preparation for studying cell volume changes in response to extracellular solute concentration changes. RBCs behave as nonideal osmometers (Solomon et al., 1986), reflecting the high concentration of hemoglobin in the cytoplasm (red cell ghosts devoid of hemoglobin display near-perfect osmotic behavior), and RBCs begin to lyse as the extracellular solute concentration is decreased, with complete lysis occurring at an extracellular osmolality of ∼100 milliosmoles/L water (Hunter, 1940), when the cell volume has increased about threefold. The RBC membrane does not support significant transmembrane pressure differences because the associated changes in membrane tension will reach the lytic membrane tension (∼10 mN/m) at a transmembrane pressure difference of ≈6 kPa, corresponding to a solute concentration difference of ≈3 milliosmoles/L water.

Nucleated cells, where the cytoskeleton extends throughout the cell, seem to better withstand osmotic challenges, and can sustain volume increases up to 10-fold over control (Stoddard et al., 1993). These initial changes in cell volume activate volume-regulatory mechanisms that catalyze the solute efflux and thereby cause the cell volume to return toward the resting volume (Hoffmann et al., 2009). In extreme cases, cells may survive incubation in distilled water for >60 min (Wan et al., 1995); even incubation in hypotonic media (osmolality reduced by 50%) produces very modest increases in membrane tension (Dai et al., 1998). Some cells possess a mechanical robustness that most likely involves the active participation of the cytoskeleton.

In this series of Perspectives, Sachs and Sivaselvan focus on the properties of a eukaryotic cell from the perspective of the cytoplasm being a poroelastic network of fibers that contribute to the mechanical robustness of eukaryotic cells. Next, Wood discusses volume regulatory mechanisms in prokaryotes, describing the channels and transporters that allow the cells to lose or gain solute and the regulation of these proteins' activity. Finally, Haswell and Verslues consider the unique problems that arise in plant cells.

Letters-to-the-editor related to these Perspectives should be received no later than June 15, 2015. The letters may be no longer than two printed pages (approximately six double-spaced pages) and will be subject to editorial review. They may contain no more than one figure, no more than 15 references, and no significant references to unpublished work. Letters should be prepared according to The Journal's Instructions and submitted at http://www.jgp.org.

REFERENCES

Dai, J., M.P. Sheetz, X. Wan, and C.E. Morris. 1998. Membrane tension in swelling and shrinking molluscan neurons. *J. Neurosci.* 18:6681–6692.

Dix, J.A., and A.S. Verkman. 2008. Crowding effects on diffusion in solutions and cells. *Annu. Rev. Biophys.* 37:247–263. http://dx.doi.org/10.1146/annurev.biophys.37.032807.125824

Finkelstein, A. 1987. Water movement through lipid bilayers, pores, and plasma membranes. Theory and reality. John Wiley, New York. 228 pp.

Hoffmann, E.K., I.H. Lambert, and S.F. Pedersen. 2009. Physiology of cell volume regulation in vertebrates. *Physiol. Rev.* 89:193–277. http://dx.doi.org/10.1152/physrev.00037.2007

Hunter, F.T. 1940. A photoelectric method for the quantitative determination of erythrocyte fragility. *J. Clin. Invest.* 19:691–694. http://dx.doi.org/10.1172/JCI101172

Luby-Phelps, K. 1999. Cytoarchitecture and physical properties of cytoplasm: Volume, viscosity, diffusion, intracellular surface area. *Int. Rev. Cytol.* 192:189–221. http://dx.doi.org/10.1016/S0074-7696(08)60527-6

Mauro, A. 1965. Osmotic flow in a rigid porous membrane. *Science.* 149:867–869. http://dx.doi.org/10.1126/science.149.3686.867

Mauro, A. 1979. Forum on osmosis. III. Comments on Hammel and Scholander's solvent tension theory and its application to the phenomenon of osmotic flow. *Am. J. Physiol.* 237:R110–R113.

Mika, J.T., and B. Poolman. 2011. Macromolecule diffusion and confinement in prokaryotic cells. *Curr. Opin. Biotechnol.* 22:117–126. http://dx.doi.org/10.1016/j.copbio.2010.09.009

Solomon, A.K., M.R. Toon, and J.A. Dix. 1986. Osmotic properties of human red cells. *J. Membr. Biol.* 91:259–273. http://dx.doi.org/10.1007/BF01868819

Stoddard, J.S., J.H. Steinbach, and L. Simchowitz. 1993. Whole cell Cl^- currents in human neutrophils induced by cell swelling. *Am. J. Physiol.* 265:C156–C165.

Vegard, L. 1908. On the free pressure in osmosis. *Proc. Camb. Philos. Soc.* 15:13–23.

Wan, X., J.A. Harris, and C.E. Morris. 1995. Responses of neurons to extreme osmomechanical stress. *J. Membr. Biol.* 145:21–31. http://dx.doi.org/10.1007/BF00233304